

Molecular Phylogeny of Eastern Pacific Porcelain Crabs, Genera *Petrolisthes* and *Pachycheles*, Based on the mtDNA 16S rDNA Sequence: Phylogeographic and Systematic Implications

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Porcelain crabs, genera *Petrolisthes* and *Pachycheles*, are diverse and abundant members of the eastern Pacific near-shore decapod crustacean community. Morphology-based taxonomic analyses of these crabs have determined groupings of affiliated species, but phylogenetic relationships remain unknown. We used sequence data from the mitochondrial 16S rRNA gene of 46 species of eastern Pacific porcelain crabs to perform phylogenetic analyses by distance and parsimony methods. Our results are used to compare the taxonomic significance of morphological and molecular characters, to examine sequence divergence rates of crab 16S rRNA genes, and to analyze the phylogeographic history of these crabs. Our phylogenetic trees indicate that the genus *Petrolisthes* is divided into two main clades, reflecting morphological features. One clade contains primarily tropical species, and the other contains species from throughout the eastern Pacific, as well as species in the genera *Allopetrolisthes* and *Liopetrolisthes*. Phylogenetic trees of *Pachycheles* suggest an antitropical distribution; north and south temperate species form one clade and tropical species form a second clade. Sequence divergence rates of the 16S rRNA gene from three pairs of geminate species can be used to date divergence times, and we discuss porcelain crab phylogeographic patterns in relation to paleogeographic events. © 2001 Academic Press

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INTRODUCTION

Porcelain crabs (family Porcellanidae) are globally distributed in temperate and tropical marine habitats and are one of the most diverse families of near-shore decapod crustaceans (Haig, 1960). The family Porcellanidae has been split into 23 genera, the largest of which are the genus *Petrolisthes*, which contains over

100 species, and the genus *Pachycheles*, which contains over 30 species (Haig, 1960; Stillman, 1998, and references therein). Approximately 45 species of *Petrolisthes* and 19 species of *Pachycheles* are found in the eastern Pacific (Haig, 1960; Stillman, 1998, and references therein). Species in these two genera are commonly found living on or among stones and corals throughout the intertidal and subtidal zones in both temperate and tropical regions. In *Petrolisthes*, sympatry is common but intertidal species generally live in discrete vertical zones (Haig, 1960; Romero, 1982; Weber Urbina, 1986; Jensen and Armstrong, 1991; Stillman, 1998). Vertical zonation creates microgeographic habitat diversity, leading to a wide variety of physiological adaptations to environmental stresses (Jensen and Armstrong, 1991; Stillman and Somero, 1996, 2000; Stillman, 1998, 2000).

The geographic distribution boundaries among porcelain crab species are sharp. There is no overlap of any porcellanid species between the eastern and the western Pacific. The eastern Pacific shares three pairs of geminate species or conspecifics with the western Atlantic that are separated by the Isthmus of Panama. These two observations suggest that the eastern Pacific porcellanid assemblage may be more similar to the western Atlantic than to the western Pacific. Such observations are common for several other taxa, including molluscs, crustaceans, and fish, and likely result from shared coancestry prior to the emergence of the Isthmus of Panama approximately 3 million years ago (Ekman, 1953; Briggs, 1974; Marshall *et al.*, 1979).

In the eastern Pacific, *Petrolisthes* and *Pachycheles* are continuously distributed from northern British Columbia to southern Chile (Haig, 1960; Carvacho, 1980). Carvacho (1980) grouped species into four biogeographic regions (Fig. 1): north temperate, south temperate, northern Gulf of California, and tropical. Between these biogeographic regions, there is no species overlap except for one species of *Petrolisthes*, *P. armatus*, which is distributed from the northern tip of the Gulf of California to Ecuador. *P. armatus* is also dis-

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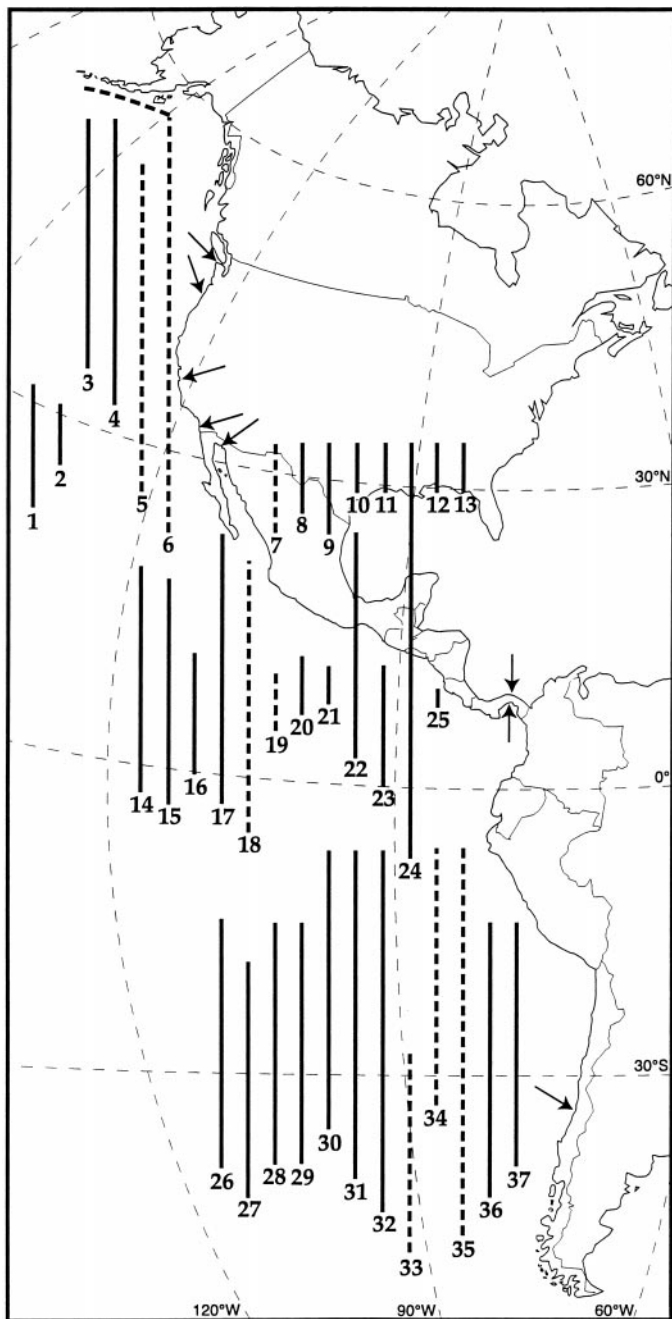


FIG. 1. Map of North and South America with approximate eastern Pacific distribution ranges of *Petrolisthes* and *Pachycheles* species used in this study. Each vertical bar represents the distribution limits for a single species, solid bars for *Petrolisthes* and dashed bars for *Pachycheles* (see Table 1 for species codes). All distributions are for the eastern Pacific coastline only except for species 7–13 and the northern limits of species 24, for which distributions are within the Gulf of California.* Arrows indicate collection locations for specimens used in this study (listed in Table 1). Distribution data from Haig (1960) and Carvacho (1980).

Note. Species 25 is only known from the Gulf of Panama in the Pacific.

tributed in the Atlantic from South Carolina to Brazil and along the western coast of Africa. The distributions of crabs in these two genera suggest that the mecha-

nisms of both vicariance and dispersal have played key roles in shaping the distribution and evolution of porcelain crabs, just as they have in many other marine taxa (Lindberg, 1991; Bowen and Grant, 1997).

In the first comprehensive examination of the eastern Pacific porcelain crabs, Haig (1960) used a qualitative analysis of morphological characters to subdivide the genus *Petrolisthes* into several groups. Haig (1960) distinguished two main groups of *Petrolisthes*: One group comprises species that have serrate spines adorning numerous parts of the exoskeleton, and the other group comprises species that are smooth or have irregular bumps or spines. Haig (1960) also suggested several subgroupings of affiliated species, but the phylogenetic affinities within the main groups of *Petrolisthes* remain unresolved.

In this study, we have performed phylogenetic analyses of a collection of species in the genera *Petrolisthes* and *Pachycheles* using DNA sequence data from a portion of the mitochondrial gene for the ribosomal large subunit (16S rRNA). 16S rDNA sequences have been successfully used to construct phylogenies in crabs (e.g., Cunningham *et al.*, 1992; Levinton *et al.*, 1996) and other arthropods (e.g., Fang *et al.*, 1993). Because *Petrolisthes* and *Pachycheles* are (i) speciose, (ii) broadly distributed, and (iii) ecologically diverse, reconstructing the phylogenetic relationships of these genera is of interest as it allows for several questions in evolution and biogeography to be addressed. First, we examine the correlations of morphology-based (Haig, 1960) and molecular-based divisions among *Petrolisthes* congeners. Second, we estimate rates of nucleotide substitution for geminate species and calibrate a molecular clock for *Petrolisthes*, thereby enabling the placement of divergence times for all clades. Such time frames for cladogenesis will allow insight into the various geologic and climatic events leading to speciation. Finally, we compare species from different geographic areas in the eastern Pacific in order to decipher historical tracks of dispersal from ancestral sources. Realistically, the number of species collected and the location of sampling sites limit this approach. However, even with an incomplete sampling of species we can begin to unravel some dispersal patterns in the eastern Pacific using a genealogical approach with approximate references to geological time.

MATERIALS AND METHODS

Sequence Acquisition

Crabs were collected from locations as indicated in Table 1 and Fig. 1 (arrows) and live specimens were identified using the dichotomous keys of Haig (1960) and Gore (1982). Specimens were frozen in liquid nitrogen and stored under cryogenic conditions until DNA extraction. Specimens were divided into taxo-

TABLE 1
Taxa Used in This Study

Species	<i>n</i>	Collection location	Map ^a	GenBank Accession No.
<i>Petrolisthes cinctipes</i>	3	Cape Arago, Oregon	3	AF260593
<i>P. eriomerus</i>	3	Cape Arago, Oregon	4	AF260595
<i>P. manimaculis</i>	3	Monterey Bay, California	1	AF260596
<i>P. cabrilloi</i>	3	La Jolla, California	2	AF260599
<i>P. granulatus</i>	3	Las Cruces, Chile	30	AF260613
<i>P. violaceus</i>	3	Las Cruces, Chile	36	AF260608
<i>P. laevigatus</i>	3	Las Cruces, Chile	32	AF260606
<i>P. tuberculatus</i>	3	Las Cruces, Chile	37	AF260607
<i>P. tuberculosus</i>	3	Las Cruces, Chile	27	AF260618
<i>P. hirtipes</i>	3	Puerto Peñasco, Mexico	9	AF260605
<i>P. crenulatus</i>	3	Puerto Peñasco, Mexico	10	AF260600
<i>P. gracilis</i>	3	Puerto Peñasco, Mexico	8	AF260601
<i>P. schmitti</i>	1	Puerto Peñasco, Mexico	12	AF260616
<i>P. tiburonensis</i>	3	Puerto Peñasco, Mexico	13	AF260603
<i>P. sanfelipensis</i>	3	Puerto Peñasco, Mexico	11	AF260604
<i>P. armatus</i> (M)	3	Puerto Peñasco, Mexico	24	AF260592
<i>P. armatus</i> (P)	3	Naos Island, Panama		AF260620
<i>P. armatus</i> (A)	3	Colón, Panama		AF260628
<i>P. cf. tridentatus</i>	3	Naos Island, Panama	23	AF260631
<i>P. tridentatus</i>	2	Colón, Panama		AF260632
<i>P. galathinus</i> var. 1	3	Naos Island, Panama	25	AF260623
<i>P. galathinus</i> var. 4	2	Colón, Panama		AF260636
<i>P. galathinus</i> var. 6	2	Colón, Panama		AF260638
<i>P. galathinus</i> var. 2	2	San Blas Islands, Panama		AF260634
<i>P. galathinus</i> var. 3	1	San Blas Islands, Panama		AF260635
<i>P. galathinus</i> var. 5	2	San Blas Islands, Panama		AF260637
<i>P. galathinus</i> var. 7	2	San Blas Islands, Panama		AF260639
<i>P. platymerus</i>	3	Naos Island, Panama	21	AF260630
<i>P. zaca</i>	3	Pacific entrance of Panama Canal, Panama	20	AF260633
<i>P. agassizii</i>	3	Naos Island, Panama	15	AF260619
<i>P. holotrichus</i>	3	Naos Island, Panama	16	AF260627
<i>P. haigae</i>	3	Naos Island, Panama	22	AF260624
<i>P. edwardsii</i>	3	Naos Island, Panama	14	AF260622
<i>P. lewisi austrinus</i>	3	Naos Island, Panama	17	AF260629
<i>Allopetrolisthes punctatus</i>	3	Las Cruces, Chile	29	AF260615
<i>A. spinifrons</i>	3	Las Cruces, Chile	28	AF260617
<i>A. angulosus</i>	3	Las Cruces, Chile	31	AF260609
<i>Liopetrolisthes mitra</i>	2	Las Cruces, Chile	26	AF260614
<i>Pachycheles pubescens</i>	1	Clallam Bay, Washington	5	AF260625
<i>Pa. rudis</i>	3	Cape Arago, Oregon	6	AF260598
<i>Pa. chilensis</i>	3	Las Cruces, Chile	33	AF260610
<i>Pa. grossimanus</i>	3	Las Cruces, Chile	35	AF260612
<i>Pa. crinimanus</i>	3	Las Cruces, Chile	34	AF260611
<i>Pa. calculosus</i>	3	Naos Island, Panama	18	AF260621
<i>Pa. trichotus</i>	3	Naos Island, Panama	19	AF260626
<i>Pa. setimanus</i>	3	Puerto Peñasco, Mexico	7	AF260602

^a Code number for eastern Pacific distribution data in Fig. 1.

onomic groupings as species except in the cases of *P. armatus* (three different populations) and *P. galathinus* and *P. tridentatus* (multiple coloration variants). Genomic DNA was extracted from muscle tissue using the Qiagen QIAamp tissue kit. DNA extractions were made from at least three different individuals from each taxonomic grouping of specimens unless fewer specimens were available, in which case multiple extractions were made such that a total of three DNA extracts were available (Table 1 contains *n* values for number of individuals sampled from each species). The

polymerase chain reaction (Mullis and Faloona, 1987; Saiki *et al.*, 1988) was used to amplify a 550-bp region of the mitochondrial 16S ribosomal gene using the primers 16SAR and 16SBR (Palumbi *et al.*, 1991; Cunningham *et al.*, 1992). Reactions consisted of 67 mM Tris-HCl, 6.7 mM MgCl₂, 16.6 mM (NH₄)₂SO₄, 0.07% β-mercaptoethanol, 0.25 mM each dATP, dGTP, dCTP, and dTTP, 2 μM primers, 0.5 units of *Taq* polymerase (Promega, Inc.), and 2 μl of a 1:50 dilution of the whole genomic DNA extraction, in a total reaction volume of 50 μl. Thermal cycling was performed in an MJ-Re-

search PTC-100 with the following conditions: 25 cycles of 94°C for 40 s, 50°C for 90 s, and 72°C for 120 s, followed by a 10-min incubation at 72°C.

PCR products were excised from a 1% TAE agarose gel and purified. Sequencing was performed using the ABI Prism Dye Terminator Cycle Sequencing kit (Applied Biosystems, Inc.) with electrophoresis on a 6% acrylamide–urea gel in an automated sequencer (ABI 373). Because sequence variation within a taxonomic grouping was low (0 to 2 mutations), these sequences were combined into a single consensus sequence for each taxonomic grouping in order to reduce the number of terminal taxa on our phylogenetic trees.

Phylogenetic Analyses

Consensus sequences were aligned using ClustalW version 1.7 (Thomson *et al.*, 1994). A total of 479 bp were aligned for analysis, and the aligned sequence set has been deposited with GenBank (see Table 1 for accession numbers). Phylogenetic analyses were conducted separately for *Pachycheles* and *Petrolisthes* because an unambiguous alignment was not possible if both genera were used. MODELTEST (Posada and Crandall, 1998) was used to select models for generation of distance matrices. PAUP* (Swofford, 1999) was used to construct distance matrix neighbor-joining trees using a maximum likelihood distance matrix for *Petrolisthes* (HKY model (Hasegawa *et al.*, 1985), proportion of invariable sites = 0.465, gamma distribution shape parameter (α) = 0.823), and a Kimura three-parameter distance matrix for *Pachycheles* (K81uf model (Kimura, 1981)). The HKY model was used because it was the closest model to the TVM+I+G model that was suggested by MODELTEST (Posada and Crandall, 1998). For both genera, PAUP* (Swofford, 1999) was used to construct parsimony trees using the heuristic search procedure. Statistical confidence was placed on nodes using the bootstrap method (Felsenstein, 1985) with 1000 replicates for all analyses. Molecular rates of nucleotide substitution were calculated using sequences from three pairs of geminate taxonomic groups from the Pacific and Atlantic coasts of Panama: *P. armatus*, *P. galathinus*, and *P. tridentatus*. To test the constancy of this molecular clock, the program PHYLTEST version 2.0 (Kumar, 1996) was used to conduct a relative rate test.

RESULTS

Phylogenetic Analyses

16S rDNA sequences were usually invariant among conspecifics, although occasionally there were polymorphisms at a single site. Among *Petrolisthes*, certain regions were difficult to align, and these data were removed in the phylogenetic analyses of the entire genus. The data removed came from two sections of the

479-bp sequence: position 20 to 26 bp and position 218 to 266 bp (from the 5' end), resulting in a final sequence length of 422 bp.

For *Petrolisthes*, distance and parsimony analyses resulted in similar tree topologies (Fig. 2). The overall tree topology suggests that the genus *Petrolisthes* is divided into two main clades: clade A containing the species *P. galathinus*, *P. sanfelipensis*, *P. edwardsii*, *P. agassizii*, *P. haigae*, *P. armatus*, and *P. zaca* and clade B containing the remaining species. Clade A can be further subdivided into additional clades (C through E). In clade B, *P. granulatus* (a south temperate species) is the most divergent lineage with bootstrap support of 83 and 93% by distance and parsimony methods, respectively. Among the remaining species in clade B, there are additional nodes that are supported, particularly one (clade F) that contains species restricted to the north temperate zone. There are a number of well-supported pairings of sister taxa within clade B, such as two species in the genus *Allopetrolisthes* (*A. angulosus* and *A. punctatus*), *P. tuberculatus* and *P. tuberculosus*, and *P. holotrichus* and *P. platymerus*. However, much of the deeper branching order within clade B is poorly resolved and bootstrap values for nodes are low.

Unambiguous alignment of *Pachycheles* 16S rDNA sequences was possible for all of the data, thus no regions were omitted during phylogenetic analyses. Sequence data from *P. galathinus* was used to root the tree (Fig. 3). Temperate species of *Pachycheles* are contained within one clade, in which the northern temperate *Pa. rudis* appears to be the most divergent taxon (Fig. 3, clade A). Two tropical species, *Pa. trichotus* and *Pa. setimanus*, are sister taxa, and *Pa. callosus* does not strongly align with any other *Pachycheles* taxa (Fig. 3).

Sequence divergence estimates were dissimilar among the three geminate pairs of species separated by the Isthmus of Panama and this caused our estimates of nucleotide substitution rate to differ (Table 2). *P. armatus* had the slowest rate (0.53%/My) while *P. galathinus* and *P. tridentatus* had rates that were twice as fast (1.36%/My and 1.03%/My, respectively). The relative rate test did not indicate a significant deviation from a constant substitution rate ($P > 0.05$) between pairs of geminate species (Table 3). These comparisons were made in an orthogonal manner using *Pachycheles pubescens* as the outgroup taxon.

DISCUSSION

Here, we have presented the first phylogenetic analyses of eastern Pacific porcelain crabs in the genera *Petrolisthes* and *Pachycheles*. Using the results of these analyses, we can begin to address some issues concerning porcelain crab systematic relationships and phylo-

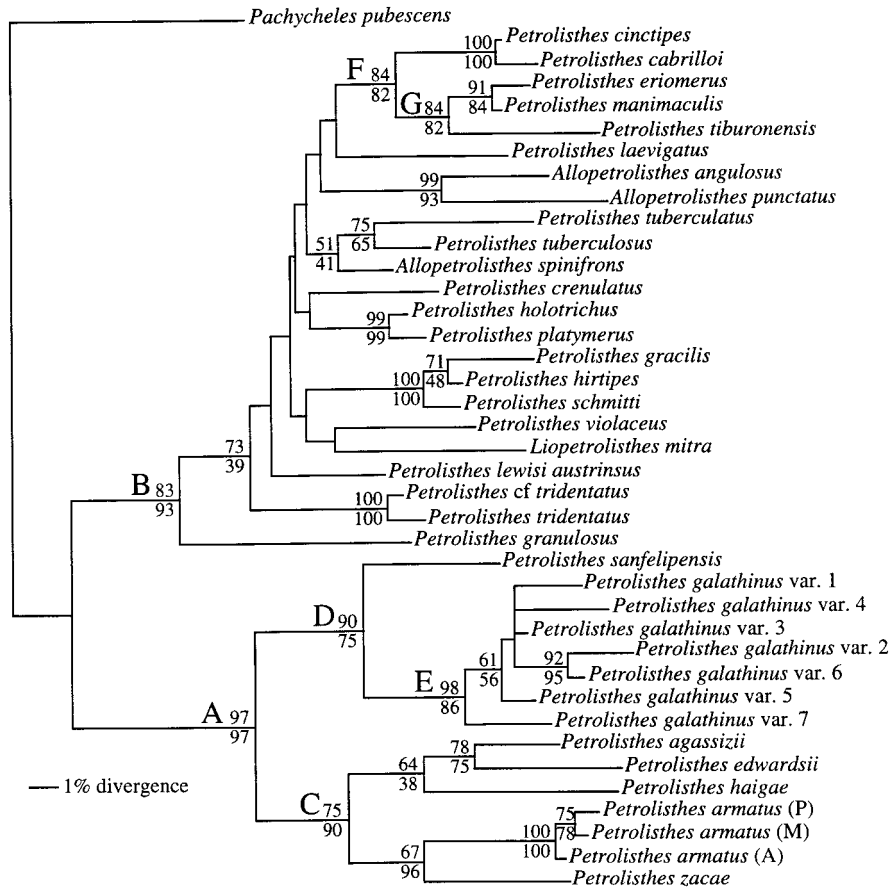


FIG. 2. Neighbor-joining distance tree for porcelain crabs in the genus *Petrolisthes* based on molecular sequence data of the 16S rRNA gene. Bootstrap values (percentages) are shown for nodes with support >50%. Upper values correspond to distance and lower values correspond to parsimony analysis. Letters denote nodes of importance as discussed in the text. The branches for *P. galathinus* variants 1, 3, and 4 were poorly supported and their branching order differed between distance and parsimony trees. They were therefore collapsed to simplify the tree. Scale bars represent 1.0% sequence divergence.

geography. We discuss our observations by genus below.

Petrolisthes

The molecular phylogenetic trees for *Petrolisthes* (Fig. 2) indicate that the genus can be subdivided into two main clades, supporting earlier division based on morphological characters (Haig, 1960). Clade A contains only species possessing regular serrate spines ("spiny") and clade B contains species that are smooth or irregularly decorated ("smooth"). Within the spiny *Petrolisthes* clade, there is a further subdivision (clades C and D) based on molecular characters. This subdivision corresponds to morphological differences in the spines on the anterior margin of the carpus of the chelipeds: species in clade D possess spines that are pronounced and numerous, while species in clade C possess fewer and less pronounced spines (spine morphology noted in Haig (1960)). The *P. galathinus* clade E contains a large amount of sequence variation com-

pared to other groups of conspecifics (7.5% among members of the *P. galathinus* clade compared to 1.6% among *P. armatus* populations). The higher sequence divergences suggest that the *P. galathinus* clade may comprise several species, subspecies, or populations.

Although morphology is very similar among all members of clade E, coloration of live specimens differs. In fact, using coloration patterns of live specimens, Werding (1986) advocated the existence of two new species (*Petrolisthes caribensis* and *P. columbiensis*) that, on the basis of morphology, were previously classified as *P. galathinus*. Comparisons of the color of live specimens used in this study with the colorations described by Werding (1986) suggest that *P. galathinus* variants 5 and 3 match *P. columbiensis*, and *P. galathinus* variants 6 and 2 match *P. caribensis*. However, only *P. galathinus* variants 6 and 2 cluster together in our phylogenetic analyses, suggesting that coloration patterns of live specimens may not be a definitive character for species identification in all

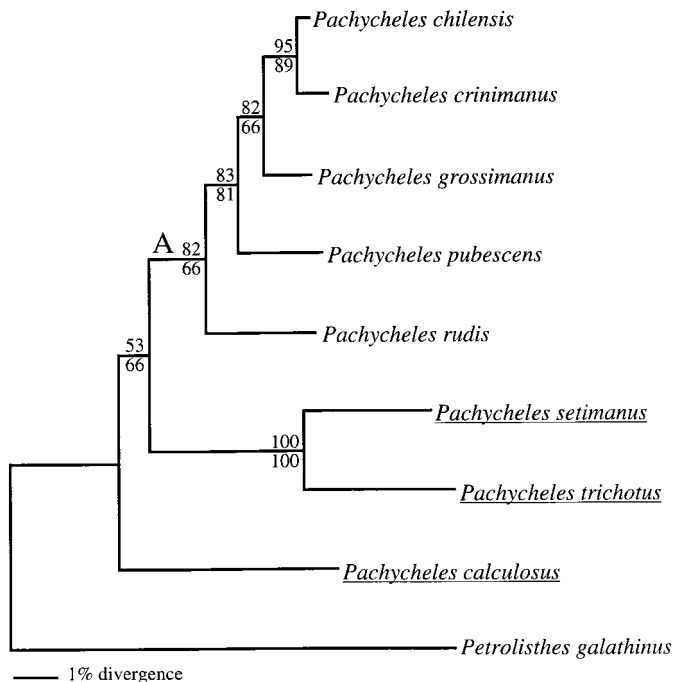


FIG. 3. Neighbor-joining distance tree for porcelain crabs in the genus *Pachycheles* based on molecular sequence data of the 16S rRNA gene. Tropical species are underlined. Bootstrap values and scale bars are as in Fig. 2.

cases. Unfortunately, we do not possess sufficient numbers of specimens for detailed fine-scale morphological examination of all of the *P. galathinus* variants. Further taxonomic work on *P. galathinus* is needed to better resolve these relationships.

The phylogenetic trees of *Petrolisthes* (Fig. 2) do not support *Allopetrolisthes* and *Liopetrolisthes* as separate genera. Instead, these taxa fall within the smooth clade of the genus *Petrolisthes*. In addition, while it is clear that *A. punctatus* and *A. angulosus* are sister taxa, *A. spinifrons* has a closer affiliation with *P. tuberculatus* and *P. tuberosus*, further questioning the cohesion of *Allopetrolisthes* as a valid genus.

The phylogenetic trees presented here allow for an

analysis of the origin of the endemic northern Gulf of California *Petrolisthes*. The Gulf of California is thought to contain a tropical fauna (Eckman, 1953; Briggs, 1974), thus we might expect the closest relatives of species endemic to the northern Gulf of California to be tropical species. Our results support this expectation, although there is also indication that some of the northern Gulf of California endemic species may have closer affiliations with temperate species (see below). Support for colonization events from the tropical eastern Pacific comes from the observations that (i) *Petrolisthes sanfelipensis* groups most closely with *P. galathinus* (Fig. 2, clade D), which is known only from Panama in the eastern Pacific, and (ii) *P. crenulatus* groups with the tropical species *P. platymerus* and *P. holotrichus*. However, statistical support for this latter grouping is low, with bootstrap values of 26 and 28% in distance and parsimony analyses, respectively. The possibility that a north temperate lineage colonized the northern Gulf of California comes from the observation that *P. tiburonensis* has its closest affiliation with four species from the northern temperate zone, *P. eriomerus*, *P. manimaculis*, *P. cinctipes*, and *P. cabrilloi* (Fig. 2, clade F). Clade F appears to have originated from temperate ancestors, thus suggesting that the taxon ancestral to *P. tiburonensis* colonized the northern Gulf of California from the north temperate. Colonization from the northern Gulf of California into the north temperate may also have occurred, as *P. eriomerus* and *P. manimaculis* speciated after *P. tiburonensis* (Fig. 2, clade G). Inclusion of the sequence data from *P. rathbunae* (the only northern temperate species of *Petrolisthes* not sampled in this study), along with the 13 tropical species missing in this data set, may strengthen hypotheses regarding the phylogenetic affiliations of northern Gulf of California *Petrolisthes*.

Pachycheles

The genus *Pachycheles* comprises one clade containing only temperate species (Fig. 3, clade A), with weak

TABLE 2

Molecular Clock Rate for the mtDNA 16S rRNA Gene from Three Geminate Species of *Petrolisthes* across the Isthmus of Panama

Species pair	% divergence ^a	Time of divergence (Mya)	Clock rate (%/My) ^b
<i>Petrolisthes armatus</i>	1.600 ± 0.0061	3.00 ^c	0.533 ± 0.002
<i>P. galathinus</i>	4.080 ± 0.0104	7.65 ^d	1.36 ± 0.0035
<i>P. tridentatus</i>	3.080 ± 0.0089	5.78 ^d	1.03 ± 0.003

^a Divergences are based on a Kimura two-parameter model with $\alpha = 0.823$ for each species pair using the complete set of sequence data available (479 bp).

^b Clock rates calculated using a divergence time of 3 My.

^c Time of divergence set at the closing of the Panamanian seaway 3 Mya (Marshall *et al.*, 1979).

^d Time of divergence based on clock rate of *Petrolisthes armatus*.

TABLE 3
Relative Rate Test Results

Species pair A	Species pair B	L_A^a	L_B^a	$L_A - L_B \pm 1 \text{ SE}$	Z score ^b	P value ^c
<i>P. galathinus</i>	<i>P. tridenatus</i>	0.118	0.145	-0.0277 ± 0.0319	0.876	>0.05
<i>P. armatus</i>	<i>P. galathinus</i>	0.094	0.078	0.0158 ± 0.0266	0.591	>0.05
<i>P. armatus</i>	<i>P. tridentatus</i>	0.125	0.137	-0.0119 ± 0.0334	0.357	>0.05

^a L_A and L_B are branch lengths to a common ancestor based on Kimura two-parameter model with $\alpha = 0.823$ and *Pachycheles pubescens* as the outgroup.

^b Z score is a two-tailed normal deviate test.

^c P value of >0.05 indicates failure to reject the null hypothesis of rate constancy.

affiliations to the remaining tropical species. An antitropical distribution pattern of temperate species is indicated in our phylogenetic trees, as northern temperate species appear to be the ancestors of the southern temperate species. Antitropical distributions of temperate species in the Pacific Ocean have been observed for several taxonomic groups (Lindberg, 1991). Eastern Pacific examples include mussels (Hilbish *et al.*, 2000), giant kelps (Estes and Steinberg, 1988), seals (King, 1983), fishes (White, 1986), and crustaceans (Dana, 1852). A study of intertidal molluscs concluded that 72% of genera were common between the Chilean and the Californian provinces (Marincovich, 1973). Previous morphologically based studies of antitropical distributions cannot rule out character convergence to explain coancestry (Lindberg, 1991). Molecular genetic data are less encumbered with this problem and can reveal antitropical distributions with greater certainty. While our sampling of tropical eastern Pacific *Pachycheles* species is incomplete, we did sample nearly all of the temperate species (3/3 south temperate and 2/3 north temperate) and the genetic similarity among temperate species relative to that among tropical species is striking. A more complete sampling of tropical eastern Pacific *Pachycheles* would allow for a verification of the antitropical distribution as well as providing evidence to suggest a mechanism that established the distribution.

Molecular Clock

Although three different substitution rates were found for the pairs of geminate *Petrolisthes* species, results from the relative rate test (Takezaki *et al.*, 1995) did not indicate an acceleration or deceleration of substitution rates along branch lengths of Atlantic/Pacific geminate species (Table 3). Thus, the differences in substitution rate more likely indicate a range of divergence times among the geminate pairs of crabs (Table 2). If *P. armatus* is used to calibrate the molecular clock, then the divergence times for *P. galathinus* and *P. tridentatus* predate the rising of the Isthmus of Panama (5 to 7 Mya). Conversely, if the rate estimated

for *P. galathinus* or *P. tridentatus* is used, the divergence time between the *P. armatus* geminate pair is from 1.2 to 1.6 Mya, long after the time (3 Mya) when the Isthmus of Panama first became a land bridge to mammals (Marshall *et al.*, 1979). Because the relative rate test did not indicate a significant deviation from equal rates of substitution in porcelain crabs, we conclude that *P. galathinus* and *P. tridentatus* likely diverged before the Isthmus of Panama arose. Thus, we suspect that *P. armatus* provides a more accurate molecular clock as its geminate species may more closely reflect a vicariant event associated with the commonly accepted geologic date used to mark the closing of the Panamanian seaway. Divergences predating the rising of the Isthmus of Panama have been suggested in other *trans-isthmian* crustaceans (Knowlton *et al.*, 1993; Knowlton and Weigt, 1998).

The 16S rDNA substitution rate estimated for *P. armatus* (0.53%/My) is close to rates observed in the mussel, genus *Mytilus* (0.54 to 0.96%/My; Rawson and Hilbish, 1995), *trans-isthmian* fiddler crabs, genus *Uca* (0.96%/My; Sturmabauer *et al.*, 1996), and *trans-isthmian* Jamaican land crabs, genus *Sesarma* (0.65%/My; Schubart, 1998). The substitution rate for *P. armatus* is also similar to that of mammals (0.2%/My; Mindell and Honeycutt, 1990). The slightly accelerated rate in crabs and mussels compared to mammals might be caused by the short (3.5 My) time frame involved in the calibration of the invertebrate clocks. To estimate the divergence times for our various clades, we believe the rate of 0.53%/My to be more reliable for recent divergences while the more conservative mammalian clock of 0.2%/My may be better suited to estimate more ancient divergences.

Phylogeographic Implications

Divergence between *Petrolisthes* spiny and smooth clades (Fig. 2, clades A and B) occurred approximately 40 to 50 Mya using the *P. armatus* clock rate, while the mammalian clock rate places this divergence at roughly 100 Mya. Clearly, estimates of the divergence times are not reliable for these nodes without a better

calibration of the molecular clock for this gene. Nonetheless, the lack of resolution in the tree (particularly in the smooth clade) among deeper lineages indicates a rapid radiation after the Cretaceous but prior to the Miocene.

The large divergence dates estimated for geminate pairs of *P. galathinus* and *P. tridentatus* may be best explained with paleontological information from the eastern Pacific. The emergence of the Panamanian Isthmus as a geographic barrier to gene flow for marine taxa likely began long before it became a bridge to terrestrial mammals (Knowlton *et al.*, 1993; Knowlton and Weigt, 1998). Holcombe and Moore (1977) have argued that the tropical circulation pattern in this region became impeded by the middle Miocene, and by the start of the Pliocene a cooler water regime was in place along the formerly warm eastern Pacific coast (Jones and Hasson, 1985). Populations of *P. galathinus* and *P. tridentatus* may have responded more immediately to climactic changes than *P. armatus*, resulting in a greater amount of genetic divergence between them. Support from this hypothesis comes from the observations that (i) populations of *P. galathinus* and *P. tridentatus* are restricted to tropical and subtropical habitats while populations of *P. armatus* extend into temperate regions (Haig, 1960) and (ii) *P. armatus* may be more physiologically eurytolerant than most other *Petrolisthes* species (Stillman, 1998; Stillman and Somero, 2000).

The molecular divergence estimated between temperate and tropical *Pachycheles* likely occurred in the early Miocene, about 20 Mya. However, divergences within the temperate clade of Chilean and Californian species did not begin until 7 to 8 Mya (Fig. 3, clade A). The geologic record shows that by the start of the Pliocene (5 Mya) microfossil faunas of Ecuador and California resemble each other (Jones and Hasson, 1985), perhaps marking the start of the antitropical distributions noted in many taxa. This information suggests that antitropical distributions in porcelain crabs might best be explained by dispersal of temperate lineages along cooler corridors that later disappeared as habitats in the eastern Pacific underwent change at the end of the Miocene. Ephemeral cool water corridors may have been responsible for trapping temperate species in the northern Gulf of California, as evidenced by *P. tiburonensis*, a taxon that groups with northern temperate species but is endemic to the northern Gulf of California. Temporary cool water corridors may also have been involved in the evolution of the temperate species *P. eriomerus* and *P. manimaculis* from *P. tiburonensis*. These temperate taxa appear to have diverged from the endemic *P. tiburonensis* (Fig. 2, clade G) approximately 14 Mya during the middle Miocene. Meanwhile, northward movement of tropical

taxa into the Gulf of California occurred roughly 16 Mya as evidenced by the divergence of *P. sanfelipensis* from *P. galathinus*. Thus migration of both temperate and tropical lineages began long before the Pliocene.

El Niño southern oscillation (ENSO) events may have played a role in establishing the biogeographic patterning of eastern Pacific porcelain crabs. Closure of the Indonesian seaway in the early to middle Miocene established a western boundary for equatorial circulation (Kennett *et al.*, 1985; MaCabe and Cole, 1989) that could assist in the formation of Kelvin surges resulting in ENSO events (Grigg and Hey, 1992). La Niña events may have followed ENSO events, causing short-term increases in upwelling intensity and thereby forming dispersal corridors for temperate species. Thus, dispersal of temperate species may have begun as soon as oceanographic conditions started to change as a result of the final closure of the circumtropical Tethys Sea. The importance of increased upwelling for dispersal of temperate near-shore eastern Pacific species is discussed in Lindberg (1991) and may be a key mechanism in explaining antitropical faunal patterns in this region.

In summary, we have reported the first molecular phylogenetic analysis of eastern Pacific porcelain crabs in the genera *Petrolisthes* and *Pachycheles*. While our sampling of species was incomplete, we were able to gain insight into the taxonomic and phylogeographic affiliations of these crabs for this first time. Our conclusions will be subject to revision as sequence data from additional species are added to the phylogenetic analyses. As porcelain crabs are broadly distributed throughout the world's oceans, there exists great potential for using these crabs in future analyses of global marine biogeography.

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