

## Does *Petrolisthes armatus* (Anomura, Porcellanidae) form a Species Complex or Are We Dealing with Just One Widely Distributed Species?

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**Fernando L. Mantelatto, Leonardo G. Pileggi, Ivana Miranda, and Ingo S. Wehrtmann (2011)** Does *Petrolisthes armatus* (Anomura, Porcellanidae) form a species complex or are we dealing with just one widely distributed species? *Zoological Studies* 50(3): 372-384. *Petrolisthes armatus* has the widest distribution known among members of the family Porcellanidae and is one of the most ubiquitous and locally abundant intertidal decapods along the Atlantic coast of the Americas. Considering its geographical distribution and morphological plasticity, several authors postulated the existence of a *P. armatus* species complex. In the present study we used genetic data from the mitochondrial 16S ribosomal gene to determine the genetic variability of *P. armatus* from selected locations within its eastern tropical Pacific and western Atlantic distributions. Our phylogenetic analysis included 49 specimens represented by 26 species of the genus *Petrolisthes* and 16 specimens from 10 species and 4 related genera. Genetic distances estimated among the analyzed *Petrolisthes* species ranged from 2.6%-22.0%; varied between 0%-5.7% for 16S. Additionally, the revision of *P. armatus* specimens from Pacific Costa Rica and Brazilian Waters showed no geographically significant morphological variations among the analyzed specimens. Therefore, our morphological and genetic data do not support the hypothesis of a *P. armatus* complex within the specimens studied herein from the Americas, but convincingly confirm the monophyly and non-separateness of the members assigned as *P. armatus*.  
<http://zoolstud.sinica.edu.tw/Journals/50.3/372.pdf>

**Key words:** Morphology, Taxonomy, Phylogenetic relationships.

Comparative studies of crustacean species known to occur over a wide geographic range can provide valuable information on the development of intraspecific adaptations to different environmental conditions (Stearns 1992, Terossi et al. 2010). Moreover, some morphological variability among closely or distantly related decapod species might be used as models to test the role of the environment in explaining evolutionary innovations by species inhabiting marine habitats. However, the number of published studies regarding this subject is limited, particularly for members of

the anomuran infraorder, which hinders the development of a more complete understanding of the evolution of life cycle strategies in this highly diverse group of crustaceans (Shirley et al. 1987, Garcia and Mantelatto 2001, Mantelatto et al. 2006, Silva et al. 2009, Weiss et al. 2010).

Porcellanid crabs represent an important part of the decapod fauna living in intertidal and shallow-water zones. Currently, there are about 279 valid species partitioned among 30 genera, primarily distributed in tropical and subtropical zones (Osawa and McLaughlin 2010). The genus

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*Petrolisthes* Stimpson, 1858 is composed of nearly 100 valid species distributed globally and is the largest genus within the family Porcellanidae (Haig 1960, Stillman and Reeb 2001, Rodríguez et al. 2005, Osawa and McLaughlin 2010).

*Petrolisthes armatus* (Gibbes 1850) has the widest distribution known in the Porcellanidae and is one of the most ubiquitous and locally abundant intertidal decapods along the Atlantic coasts of the Americas. Its known distribution covers wide ranges on both the Atlantic and Pacific coasts (Melo 1999, Stillman and Reeb 2001): in the western Atlantic Ocean, it ranges from North Carolina to Florida, the Gulf of Mexico, the Antilles, Central America, Venezuela, Colombia, and Brazil (Rodríguez et al. 2005); in the eastern Atlantic from Senegal to Angola, and Ascension I.; and in the eastern Pacific, from the Gulf of California to Costa Rica, the Galapagos I., and Peru. Its wide geographical distribution makes this species an ideal and frequently used candidate for studies on distribution and biogeography (Carvacho 1980, Gore 1982, Stillman and Somero 2000, Werding et al. 2003, Rodríguez et al. 2005), molecular phylogeny (Stillman and Reeb 2001, Morrison et al. 2002), population dynamics (Oliveira and Masunari 1995, Díaz-Ferguson and Vargas-Zamora 2001, Miranda and Mantelatto 2009), and reproduction (Wehrtmann et al. 2011).

The apparent distribution of a species over a wide geographical range may raise the question of whether there is one or several, morphologically similar species. As an example involving decapods, many studies focusing on the genetic differentiation of different lobster populations led to the recognition of subspecies or new species (Sarver et al. 1998 2000, García-Rodríguez and Perez-Enriquez 2008). Considering the wide geographical distribution and morphological plasticity reported for *P. armatus*, it is not surprising that several authors mentioned the possibility of a *P. armatus* species complex (Rodríguez et al. 2005, Hiller et al. 2006).

Therefore, in the present study, we used genetic data from the mitochondrial 16S ribosomal gene to determine the genetic variability of *P. armatus* from the eastern tropical Pacific (Costa Rica) and the western Atlantic distributions (Costa Rica, Panama, Venezuela, and Brazil). Additionally, morphological data were compiled from Haig (1960), and the encountered diagnostic differences were compared with our molecular findings. Our results provide solid evidence to reject the hypothesis of a *P. armatus* species

complex within the specimens studied herein from the Americas.

## MATERIAL AND METHODS

### Sample collection

Porcellanid crabs were collected in 2004–2009 from different localities or were provided by colleagues (Table 1). Freshly collected specimens were directly preserved in 75%–90% ethanol. Species identification was confirmed using morphological characters described in the literature (Haig 1960, Melo 1999). The following morphological characters were checked: carapace ornamentation (granulations or plications), the presence of an epibranchial spine, front shape, the number and shape of the teeth on the carpus of the chelipeds, walking legs on the anterior margin and presence of a spine on the posterodistal angle of 1st and 2nd legs. Specimen vouchers from which new tissue subsamples were obtained were deposited in the Crustacean Collection of the Biology Department (CCDB), Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), Univ. of São Paulo (USP) (Table 1).

### DNA extraction, amplification, and sequencing

The sequences used in this study were generated from our own extractions (14 sequences) or retrieved from GenBank (Table 1). When possible, 2–5 specimens from each collection site were used for the species analyses to limit the chance of misidentifications and also to observe the variability. Apart from *P. armatus*, we included other porcellanid crabs from other genera to compare them with a broader extension of the phylogenetic tree.

DNA extraction, amplification, and sequencing protocols followed Schubart et al. (2000a) with modifications described by Mantelatto et al. (2006 2007 2009a) and Robles et al. (2007). Total genomic DNA was extracted from muscle tissue from the walking legs or chelipeds. The muscle was ground up and incubated for 1–12 h in 600  $\mu$ l of lysis buffer at 65°C; proteins were separated by the addition of 200  $\mu$ l of 7.5 M ammonium acetate prior to centrifugation. DNA precipitation was achieved by the addition of 600  $\mu$ l of cold isopropanol followed by centrifugation; the resultant pellet was washed with 70% ethanol, dried, and resuspended in 10–20  $\mu$ l of TE buffer.

An approximately 550-base pair (bp) region of the 16S rDNA gene was amplified from diluted DNA by means of a polymerase chain reaction (PCR) conducted in a Thermo® PxE 0.2 Thermal Cycler (Waltham, MA, USA) with the following thermal cycles: initial denaturing for 10 min at 94°C, annealing for 38-42 cycles of 1 min at 94°C, 1 min at 45-48°C, and 2 min at 72°C, with a final extension of 10 min at 72°C. The primers were designated as follows: 16SH2 (5'-AGATAGAAACCAACCTGG-3'), 16SL2 (5'-TGCCTGTTTATCAAAAACAT-3'), and 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') (for references on the primers see Schubart et al. 2000a b). PCR products were purified using a SureClean Plus kit (Bioline Inc., USA) and sequenced with ABI Big Dye® Terminator Mix in an ABI Prism 3100 Genetic Analyzer® following Applied Biosystems protocols (Applied Biosystems, Carlsbad, CA, USA). All sequences were confirmed by sequencing both strands. A consensus sequence for the 2 strands was obtained using BioEdit vers. 7.0.7.1 (Hall 2005).

### Phylogenetic analysis

Sequences were edited with the BioEdit program (Hall 2005). The phylogenetic analysis was exclusively based on a partial fragment of the 16S rDNA gene. We carried out a dynamic analysis using POY software, vers. 4 (Varón et al.

2010), under direct optimization with parsimony as the optimality criterion (Wheeler 1996). Trees were constructed through a random addition sequence followed by a combination of branch-swapping steps (subtree pruning and regrafting (SPR) and tree bisection and reconnection (TBR)). The ratcheting procedure was used to enhance branch swapping by randomly reweighting characters during the SPR and TBR procedures. A sensitivity analysis was carried out using different cost matrices (Wheeler 1996). All datasets were analyzed under 10 parameter sets for the range of indel, transition, and transversion ratios (Table 2).

### Divergence analysis

Sequences were aligned using ClustalX (Thompson et al. 1997) with an interface in BioEdit with default parameters (Hall 2005). Ambiguous regions in the alignment were removed. Sequences were analyzed using the Modeltest program (Posada and Crandall 1998) to find the evolutionary model that best fits the data. Phylogenetic reconstructions were conducted using PAUP 4.0 beta 10 (Swofford 2003) for the distance analysis (minimum evolution). The consistency of the topologies was measured by the bootstrap method (with 1000 bootstraps), and only confidence values of > 50% are reported. To evaluate the range of intrageneric sequence identity found among recognized species, we

**Table 1.** Porcellanid crab species used for the molecular phylogenetic reconstructions with respective site of collection, museum catalogue number, and genetic database accession numbers (GenBank) (CCDB, Crustacean Collection of the Department of Biology, FFCLRP, Univ. of São Paulo, Ribeirão Preto, Brazil; CNCR, Colección Nacional de Crustáceos, Instituto de Biología, Univ. Nacional Autónoma de México, Mexico D.F., Mexico; MZUESC, Museum of Zoology, Univ. of Santa Cruz, Bahia, Brazil; ULLZ, Zoological Collection, Univ. of Louisiana at Lafayette, Lafayette, LA, USA)

Species	Collection site	Catalogue no.	GenBank accession no.
<i>Megalobrachium poeyi</i> (Guérin, 1855)	Quintana Roo (Mexico)	CNCR 9818	DQ865326
<i>Megalobrachium poeyi</i> (Guérin, 1855)	Isla Margarita (Venezuela)	ULLZ 5343	DQ865327
<i>Megalobrachium roseum</i> (Rathbun 1900)	Ubatuba, SP (Brazil)	CCDB 2854	HM352468
<i>Megalobrachium soriatum</i> (Say, 1818)	Florida (USA)	ULLZ 5262	DQ865325
<i>Neopisosoma angustifrons</i> (Benedict, 1901)	Vera Cruz (Mexico)	ULLZ 5373	DQ865336
<i>Neopisosoma angustifrons</i> (Benedict, 1901)	Isla Cubagua (Venezuela)	ULLZ 5345	DQ865337
<i>Pachycheles ackleianus</i> A. Milne-Edwards, 1880	Looe Key Reef (Florida)	ULLZ 4824	DQ865332
<i>Pachycheles grossimanus</i> (Guérin, 1835)	Valdivia (Chile)	CCDB 2105	HM352466
<i>Pachycheles monilifer</i> (Dana, 1852)	Ubatuba, SP (Brazil)	CCDB 696	HM352467
<i>Pachycheles monilifer</i> (Dana, 1852)	Vera Cruz (Mexico)	ULLZ 5388	DQ865330
<i>Pachycheles monilifer</i> (Dana, 1852)	Isla Cubagua (Venezuela)	ULLZ 5348	DQ865331
<i>Pachycheles pilosus</i> (H. Milne Edwards, 1837)	Quintana Roo (Mexico)	ULLZ 5389	DQ865328

**Table 1.** (Continued)

Species	Collection site	Catalogue no.	GenBank accession no.
<i>Pachycheles pilosus</i> (H. Milne Edwards, 1837)	Isla La Tortuga (Venezuela)	ULLZ 5349	DQ865329
<i>Pachycheles susanae</i> Gore and Abele, 1973	Quintana Roo (Mexico)	CNCR 7273	DQ865333
<i>Pachycheles susanae</i> Gore and Abele, 1973	Isla Cubagua (Venezuela)	ULLZ 5352	DQ865334
<i>Petrolisthes amoenus</i> (Guérin, 1855)	Isla Cubagua (Venezuela)	Unknown	DQ444873
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Caravelas, BA (Brazil)	MZUESC 975	HM352470
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Nova Viçosa, BA (Brazil)	MZUESC 834	HM352471
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Paraty, RJ (Brazil)	CCDB 2314	HM352472
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Florianópolis, SC (Brazil)	CCDB 1834	HM352473
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Itajaí, SC (Brazil)	CCDB 1883	HM352474
<i>Petrolisthes armatus</i> (Gibbes, 1850)	São Sebastião, SP (Brazil)	CCDB 1747	HM352475
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Ubatuba, SP (Brazil)	CCDB 2315	HM352476
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Punta Morales (Pacific Costa Rica)	CCDB 1718	HM352477
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Salinas (Ecuador)	Unknown	DQ444874
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Bocas del Toro (Panama)	CCDB 2582	HM352478
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Isla Margarita (Venezuela)	CCDB 1819	HM352479
<i>Petrolisthes armatus</i> (Gibbes, 1850)	Florida (USA)	ULLZ 5252	DQ865310
<i>Petrolisthes agassizii</i> Faxon 1893	Gorgona I. (Colombia)	Unknown	DQ444872
<i>Petrolisthes bolivarensis</i> Werding and Kraus, 2003	San Bernardo Is. (Colombia)	Unknown	DQ444876
<i>Petrolisthes caribensis</i> Werding, 1983	Twin Cays (Belize)	Unknown	DQ865295
<i>Petrolisthes caribensis</i> Werding, 1983	Quintana Roo (Mexico)	Unknown	DQ865297
<i>Petrolisthes columbiensis</i> Werding, 1983	Islas del Rosário (Colombia)	Unknown	DQ444899
<i>Petrolisthes edwardsii</i> (Saussure, 1853)	Salinas (Ecuador)	Unknown	DQ444904
<i>Petrolisthes galathinus</i> (Bosc, 1801)	Carrie Bow (Belize)	Unknown	DQ865299
<i>Petrolisthes galathinus</i> (Bosc, 1801)	Salinas (Ecuador)	Unknown	DQ444933
<i>Petrolisthes galathinus</i> (Bosc, 1801)	Quintana Roo (Mexico)	CNCR 10937	DQ865303
<i>Petrolisthes galathinus</i> (Bosc, 1801)	Estero Aserradores (Nicaragua)	ULLZ 5316	DQ865302
<i>Petrolisthes galathinus</i> (Bosc, 1801)	Isla Cubagua (Venezuela)	ULLZ 5355	DQ865301
<i>Petrolisthes glasselli</i> Haig, 1957	Isla Malpelo (Colombia)	Unknown	DQ444939
<i>Petrolisthes haigae</i> Chace, 1962	Naos I. (Panama)	Unknown	AF260624
<i>Petrolisthes haigae</i> Chace, 1962	Salinas (Ecuador)	Unknown	DQ444940
<i>Petrolisthes hirtispinosus</i> Lockington, 1878	Isla Angel de la Guarda (Mexico)	Unknown	DQ444941
<i>Petrolisthes jugosus</i> Streets, 1872	Quintana Roo (Mexico)	ULLZ 5406	DQ865315
<i>Petrolisthes lewisi</i> (Glassell, 1936)	Estero Nagualapa (Nicaragua)	ULLZ 5321	DQ865317
<i>Petrolisthes magdalenensis</i> Werding, 1978	Isla Margarita (Venezuela)	ULLZ 5358	DQ865316
<i>Petrolisthes marginatus</i> Stimpson, 1859	Veracruz (México)	ULLZ 5410	DQ865308
<i>Petrolisthes marginatus</i> Stimpson, 1859	Quintana Roo (Mexico)	ULLZ 5412	DQ865306
<i>Petrolisthes monodi</i> Chace, 1956	Cape Verde	Unknown	DQ444944
<i>Petrolisthes politus</i> (Gray, 1831)	Quintana Roo (Mexico)	ULLZ 5412	DQ865306
<i>Petrolisthes politus</i> (Gray, 1831)	Isla Cubagua (Venezuela)	ULLZ 5360	DQ865307
<i>Petrolisthes quadratus</i> Benedict, 1901	Quintana Roo (Mexico)	CNCR 3724	DQ865318
<i>Petrolisthes quadratus</i> Benedict, 1901	Isla La Tortuga (Venezuela)	ULLZ 5361	DQ865319
<i>Petrolisthes robsonae</i> Glassell, 1945	Darien (Panama)	Unknown	DQ444946
<i>Petrolisthes rosariensis</i> Werding, 1978	Carrie Bow Cay (Belize)	Unknown	DQ865305
<i>Petrolisthes sanfelipensis</i> Glassell, 1936	Puerto Penasco (Mexico)	Unknown	AF260604
<i>Petrolisthes sanmartini</i> Werding and Hiller, 2002	Islas del Rosario (Colombia)	Unknown	DQ444954
<i>Petrolisthes tonsorius</i> Haig, 1960	Cabo Corrientes (Colombia)	Unknown	DQ444959
<i>Petrolisthes tridentatus</i> Stimpson, 1859	Quintana Roo (Mexico)	ULLZ 5414	DQ865322
<i>Petrolisthes tridentatus</i> Stimpson, 1859	Isla Margarita (Venezuela)	ULLZ 5363	DQ865323
<i>Petrolisthes tridentatus</i> Stimpson, 1859	Estero Nagualapa (Nicaragua)	ULLZ 5324	DQ865321
<i>Petrolisthes tridentatus</i> Stimpson, 1859	Isla Naos (Panama)	ULLZ 5364	DQ865320
<i>Petrolisthes violaceus</i> (Guérin, 1831)	Coquimbo (Chile)	CCDB 2109	HM352469
<i>Petrolisthes zacaе</i> Haig, 1968	Bahia Malaga (Colombia)	Unknown	DQ444962
<i>Porcellana sayana</i> (Leach, 1820)	Isla Margarita (Venezuela)	ULLZ 5366	DQ865339

compared genetic distances between species pairs by pairwise comparisons using *p*-distances as the substitution model in the PAUP program. The distance matrix shows the proportion of divergent residues among all sequences in the alignment as they were currently aligned.

## RESULTS

### Taxonomic assignments

Based on the morphology, voucher materials were assigned to species as follows, and DNA was successfully sequenced for those underlined (CW, carapace width).

#### ***Petrolisthes armatus* (Gibbes, 1850)**

*Porcellana armata* Gibbes, 1850: 190 (type locality, Florida, USA), Not *P. armata* Dana, 1852: 426.

*Petrolisthes armatus* (see Haig 1960 for citations under this date), Gore 1970: 75, 76, 79, 86, 87, figs. 1-6; Gore 1972: 67, 71, 79, 80, 82, figs. 1-6; Werding 1977: 176, 198, fig. 18; Hendrickx and Harvey 1999: 379; Stillman and Reeb 2001: 236, 238-243, fig. 2; Werding et al. 2003: 79, 81, 82; Vargas and Cortés 2006: 463, 470; Hiller et al. 2006: 6, 10, 12-14, 19, 20; Rodríguez et al. 2005: 544, 545, 559, 560, 571, 572; Rodríguez et al. 2006: 115, 116, 118, 123, 124, 126; Coelho et al. 2007: 12.

**Materials examined:** 2 ♂♂ (7.9, 9.6\* mm CW), 4 ♀♀ (5.2, 5.7, 7.2, 7.8 mm CW), Playa Curu, Paquera, Pacific Costa Rica, Feb. 2009, CCDB 2581; 2 ♂♂ (11.6, 12.7\* mm CW), 1 ♀ (9.6 mm CW), Playa Blanca, Punta Morales, Pacific Costa Rica, Sept. 2005, CCDB 1718; 3 ♀♀ (4.7, 5.40, 7.0\* mm CW), Playa Bluff, Panama, Feb. 2009, CCDB 2582; 1 ♂ (8.0\* mm CW), Muelle de la Guardia, Isla Margarita, Venezuela, Aug. 2006, CCDB 1819; 1 ♀ (9.8\* mm CW), Playa Valdez, Isla Margarita, Venezuela, Aug. 2006, CCDB 1811; 5 ♂♂ (3.5, 3.8, 4.5, 5.0, 6.0\* mm CW), 4 ♀♀ (2.4, 3.5, 4.3, 6.2 mm CW), Rio Caravelas, Caravelas (BA), Brazil, Aug. 2007, MZUESC 975; 3 ♂♂ (2.5, 4.6, 10.8\* mm CW), 2 ♀♀ (4.4, 4.6 mm CW), Praia Pontal da Barra, Nova Viçosa (BA), Brazil, Mar. 2007, MZUESC 834; 1 ♂ (mm CW), Guarapari Channel (ES), Brazil, Nov. 2006, CCDB 2583; 2 ♂♂ (3.6, 7.2 mm CW), 3 ♀♀ (4.1, 6.0, 7.3\* mm CW), Paraty (RJ), Brazil, Aug. 2007, CCDB 2314; 1 ♂ (12.5 mm CW), Praia Grande, Ubatuba (SP), Brazil, June 2008, CCDB 2377; 1 ♂ (10.9 mm CW), 2 ♀♀ (7.4, 10.2 mm CW), Saco do Codó, Ubatuba (SP), Brazil, June 2008, CCDB 2369; 1 ♂ (13.6\* mm CW), Praia

do Pinto, Ubatuba (SP), Brazil, Nov. 2006, CCDB 2315; 4 ♂♂ (8.6, 8.7, 10.0, 10.8 mm CW), 2 ♀♀ (7.0, 9.7 mm CW), Praia do Araçá, São Sebastião (SP), Brazil, July 2006, CCDB 1844; 2 ♂♂ (9.0\*, 11.6 mm CW), 1 ♀ (11.6 mm CW), Channel 6, Santos (SP), Brazil, July 2003, CCDB 2583; 2 ♂♂ (5.7\*, 7.0 mm CW), 1 ♀ (7.6 mm CW), Praia da Ponta do Sambaqui, Florianópolis (SC), Brazil, Apr. 2007, CCDB 1894; 1 ♂ (7.7 mm CW), 2 ♀♀ (7.0, 9.1\* mm CW), Praia dos Ingleses, Bombinhas (SC), Brazil, Apr. 2007, CCDB 1895.

**Diagnosis:** Carapace granulate and plicate; a single epibranchial spine present, occasionally obsolescent; front sinuously triangular; carpus 2-2.5 times as long as wide, anterior margin with 3 low, widely set teeth, a 4th occasionally present; manus rather long and slender; merus of walking legs with a row of spines on anterior margin, that of legs 1- and 2-spined at posterodistal angle.

**Description:** Carapace naked or pubescent, covered with granules and short, rough plications; about as broad in mid-branchial regions as posteriorly. Front broadly, sinuously triangular, sometimes pubescent, with a deep median sulcus; no supraocular spine. Outer orbital angle not produced. A single epibranchial spine, occasionally obsolescent. First moveable antenna with a strongly projecting, lamellar lobe, usually spine-tipped; 2nd with a large projecting tubercle; 3rd nearly smooth, flagellum naked. Outer maxillipeds rugose. Inferior margin of side walls with a fringe of plumose hairs. Merus of chelipeds rugose, armed on anterior with a large lobe, usually spine-tipped. Carpus 2-2.5-times as long as wide, naked or pubescent, especially on anterior margin; covered with large granules tending to form a median longitudinal crest, especially in young specimens; anterior margin armed with 3 low, widely set, spine-tipped teeth, a 4th occasionally present; posterior margin with a row of large flattened granules, with 1-4 produced into spines, and ending distally in a large bifid spine. Manus narrow, naked or pubescent, especially on proximal margin, covered with scattered granules similar to those of carpus; outer margin sometimes with a row of short spines and a fringe of hairs, particularly in young specimens. External margin of dactylus with a well-defined sulcus from basis to tip, on both chelipeds. Gape of fingers with short pubescence. Walking legs rugose; anterior margin of merus and carpus with plumose hairs, all segments with long, non-plumose setae; anterior margin of merus of leg 1 with 1-5 spines, of leg 2 with 2-6, of leg 3 with 0-3; merus of legs 1 and 2

with 1 or 2 posterodistal spines, 1 also occasionally present on leg 3.

**Remarks:** According to the original description, the merus of pereopod 1 presents between 2-5 spines, but specimens from southeastern Brazil (Rio de Janeiro and São Paulo States) may present only 1 spine, extending the range of variation of this character.

### Phylogenetic analysis

Our phylogeny included 65 specimens representing 26 species of the genus *Petrolisthes* and 16 specimens from 10 species and 4 related genera. In total, we generated 14 new partial 16S sequences (~550 bp).

Of the 10 parameter sets analyzed by direct optimization, the one that produced the shortest trees was that for an indel/transition/transversion ratio of 1:1:1 (parameter set 111, Table 2). The parsimony analysis yielded a single most-parsimonious tree of length 1328 (Fig. 1). The analysis showed strong consistency, well supported by the bootstrap and sensitivity analyses, in most nodes that included taxa from

the Pacific and Atlantic regions in the same clade. The most-parsimonious tree (Fig. 1) showed the non-separation of the species *P. armatus* in a complex.

### Genetic divergence

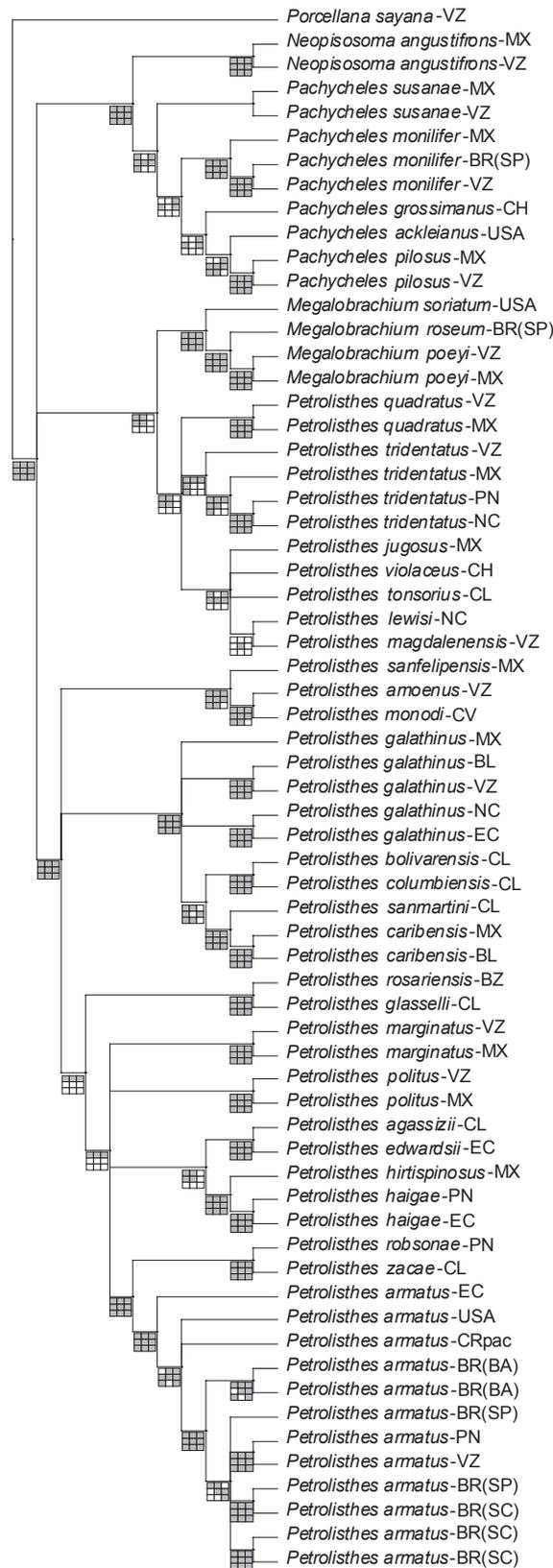
The multiple sequence alignment for the 16S gene had 550 positions for 49 *Petrolisthes* specimens and 16 from other related genera. In general, the dendrogram generated by the cluster analysis presented the same topology as the phylogenetic analysis (Fig. 2).

Genetic distances estimated among the *Petrolisthes* species analyzed herein ranged from 2.6% (*P. robsonae*-PN and *P. zacae*-CL) to 22.0% (*P. violaceus*-CH and *P. marginatus*-VZ and MX) (data not shown); intraspecific divergences ranged from 0 (*P. tridentatus*-VZ) to 5.7% (*P. tridentatus*-MX) for 16S (data not shown).

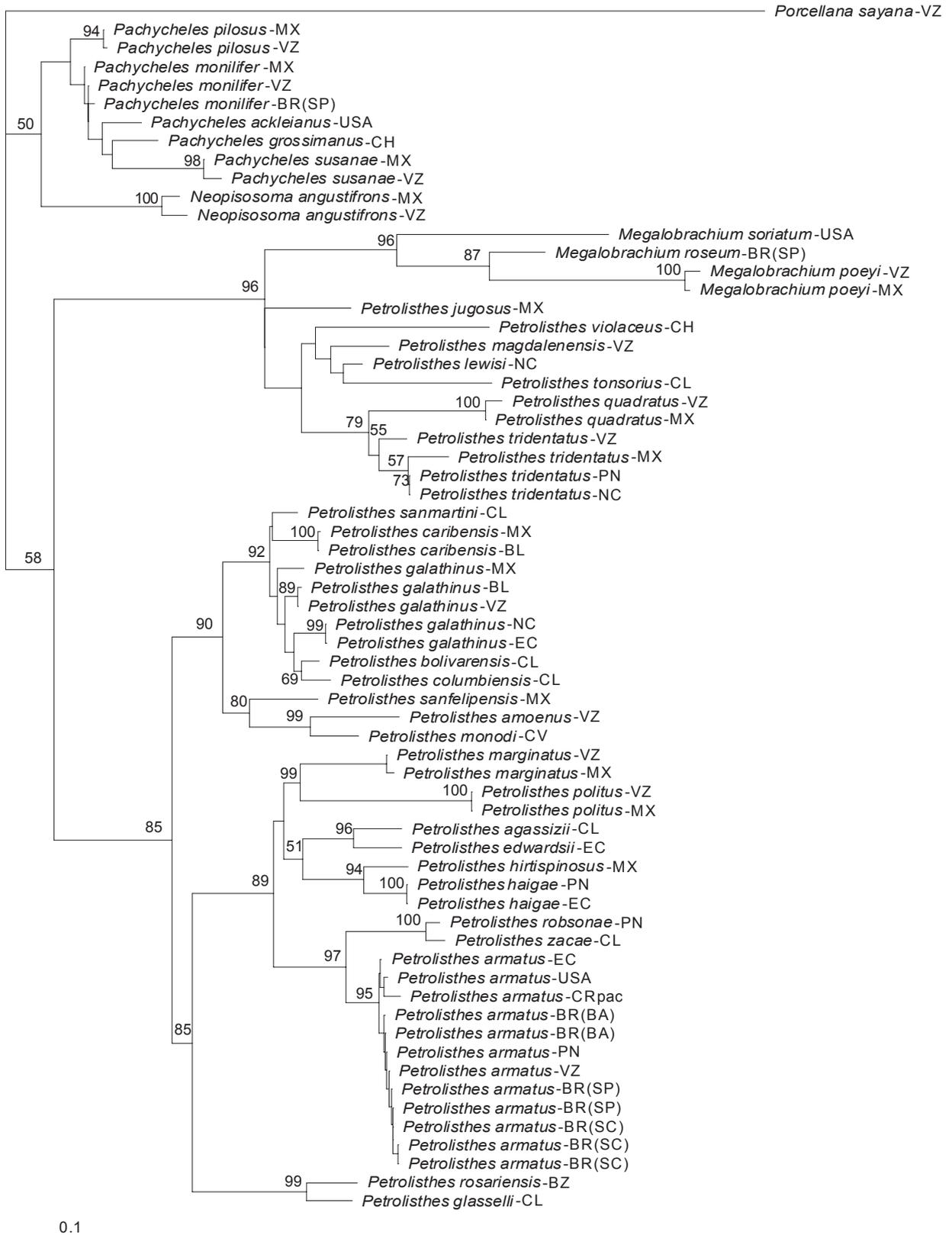
Positional differences between putative populations of *P. armatus* were very limited, with a genetic divergence that ranged from 0%-2.1% for the 16S gene (Table 3). Even so, genetic divergences among Atlantic and Pacific

**Table 2.** Matrices used in the sensitivity analysis. The numbers correspond to the ratio of indel/transition/transversion values

<p>Matrix 111</p> <p>A C G T -</p> <p>A 0 1 1 1 1</p> <p>C 1 0 1 1 1</p> <p>T 1 1 0 1 1</p> <p>G 1 1 1 0 1</p> <p>- 1 1 1 1 0</p>	<p>Matrix 112</p> <p>A C G T -</p> <p>A 0 2 1 2 1</p> <p>C 2 0 2 1 1</p> <p>T 1 2 0 2 1</p> <p>G 2 1 2 0 1</p> <p>- 1 1 1 1 0</p>	<p>Matrix 113</p> <p>A C G T -</p> <p>A 0 3 1 3 1</p> <p>C 3 0 3 1 1</p> <p>T 1 3 0 3 1</p> <p>G 3 1 3 0 1</p> <p>- 1 1 1 1 0</p>	<p>Matrix 211</p> <p>A C G T -</p> <p>A 0 1 1 1 2</p> <p>C 1 0 1 1 2</p> <p>T 1 1 0 1 2</p> <p>G 1 1 1 0 2</p> <p>- 2 2 2 2 0</p>
<p>Matrix 212</p> <p>A C G T -</p> <p>A 0 2 1 2 2</p> <p>C 2 0 2 1 2</p> <p>T 1 2 0 2 2</p> <p>G 2 1 2 0 2</p> <p>- 2 2 2 2 0</p>	<p>Matrix 221</p> <p>A C G T -</p> <p>A 0 1 2 1 2</p> <p>C 1 0 1 2 2</p> <p>T 2 1 0 1 2</p> <p>G 1 2 1 0 2</p> <p>- 2 2 2 2 0</p>	<p>Matrix 411</p> <p>A C G T -</p> <p>A 0 1 1 1 4</p> <p>C 1 0 1 1 4</p> <p>T 1 1 0 1 4</p> <p>G 1 1 1 0 4</p> <p>- 4 4 4 4 0</p>	<p>Matrix 412</p> <p>A C G T -</p> <p>A 0 2 1 2 4</p> <p>C 2 0 2 1 4</p> <p>T 1 2 0 2 4</p> <p>G 2 1 2 0 4</p> <p>- 4 4 4 4 0</p>
<p>Matrix 812</p> <p>A C G T -</p> <p>A 0 2 1 2 8</p> <p>C 2 0 2 1 8</p> <p>T 1 2 0 2 8</p> <p>G 2 1 2 0 8</p> <p>- 8 8 8 8 0</p>	<p>Matrix 821</p> <p>A C G T -</p> <p>A 0 1 2 1 8</p> <p>C 1 0 1 2 8</p> <p>T 2 1 0 1 8</p> <p>G 1 2 1 0 8</p> <p>- 8 8 8 8 0</p>		



**Fig. 1.** Phylogenetic tree of selected species of *Petrolisthes* and related groups, based on direct optimization analysis of 16S rDNA datasets under the parameter set which produced the most-parsimonious tree. The box at the bottom left indicates the parameter sets used in the analysis. Filled boxes signify that the clade was present.



**Fig. 2.** Dendrogram of the cluster analysis for species of *Petrolisthes* and related groups, based on a distance analysis using the minimum evolution (ME) algorithm of 16S rDNA gene sequences. Numbers below are significance values for 1000 bootstraps; values  $\leq 50\%$  are not shown.

populations were generally more pronounced than those among Atlantic populations. Specimens from Ecuador showed proximity to specimens from the Costa Rican Pacific and surprisingly to specimens from the USA (Gulf of Mexico). Among the Atlantic populations, the genetic variability was low, showing proximity between populations of Panama and Venezuela to those of Brazil (Bahia, São Paulo, and Santa Catarina).

## DISCUSSION

The present investigation, based on analyses of morphology and a partial fragment of 16S rDNA, supported the monophyly of *P. armatus*. While we acknowledge the general phylogenetic proximity of these specimens, there is no genetic justification for a separation into different species, rejecting the hypothesis of the possible existence of a *P. armatus* complex mentioned in the literature (Rodríguez et al. 2005, Hiller et al. 2006). However, our data were exclusively generated by material from the Americas, and additional genes and material covering the entire geographic range is necessary to allow a more-comprehensive treatment of *P. armatus*.

Although *Petrolisthes* is the largest genus within the family Porcellanidae, it is recognized that species tend to fall into several natural groups, some of which were previously discussed by Ortmann (1897) (see Haig 1960). Throughout the years, *Petrolisthes* has been discussed because interpretations of species interrelationships were ambiguous. However, specialists recommended that any attempt at splitting the genus should be

postponed until a worldwide basis for this genus is available and can be studied, especially from a phylogenetic point of view (Haig 1960, Stilmann and Reeb 2001, Rodríguez et al. 2006).

In our set of analyzed species (26 of 100 recognized species), *Petrolisthes* can be subdivided into 2 main clades, supporting an earlier division based on morphological characters (Haig 1960) and corroborating the subdivision proposed by Stillman and Reeb (2001) that named the 2 groups "smooth" and "spiny". Clade A (group I or "smooth" ) contains *P. violaceus* Guérin 1831, the type species of the genus, and other species that share the following morphological features: a smooth carapace, the absence of an epibranchial spine, and the merus of the pereopods without spines. This group includes some species of the genus *Megalobrachium* Stimpson 1858; the adult forms are more similar to the genera *Polyonyx* Stimpson 1858, *Minyocerus* Stimpson 1858, *Pisidia* Leach 1820, and *Porcellana* Lamarck 1801, in which the basal antennal article is strongly produced forward and broadly contacts the margin of the carapace, so that the moveable part is far removed from the orbit. On the other hand, *Megalobrachium* larvae share characteristics similar to the genera *Petrolisthes*, *Pachycheles* (Stimpson 1858), *Neopisosoma* Haig 1960, *Allopetrolisthes* Haig 1960, and *Clastoetochus* Haig 1960; these features are not found in larvae of the genera *Polyonyx*, *Minyocerus*, *Pisidia*, or *Porcellana* (Gore 1970 1973, Hernandez et al. 2002). According to Rodríguez et al. (2006), *Megalobrachium* constitutes a distinct clade from *Petrolisthes*, and presents a very high molecular divergence which could argue in favor

**Table 3.** Genetic divergent matrix of the 16S gene among *Petrolisthes armatus* specimens. (USA = United States of America; CRpac = Costa Rica, Pacific coast; EC = Ecuador; PAN = Panama; VZ = Venezuela; BR(SP) = Brazil, São Paulo; BR(SC) = Brazil, Santa Catarina; BR(BA) = Brazil, Bahia)

<i>P. armatus</i> -USA	0												
<i>P. armatus</i> -CRpac	0.015	0											
<i>P. armatus</i> -EC	0.017	0.008	0										
<i>P. armatus</i> -PAN	0.015	0.011	0.015	0									
<i>P. armatus</i> -VZ	0.015	0.011	0.015	0.000	0								
<i>P. armatus</i> -BR(SP)	0.019	0.015	0.019	0.000	0.000	0							
<i>P. armatus</i> -BR(SP)	0.021	0.017	0.021	0.002	0.002	0.002	0						
<i>P. armatus</i> -BR(SC)	0.021	0.017	0.021	0.002	0.002	0.002	0.000	0					
<i>P. armatus</i> -BR(SC)	0.021	0.017	0.021	0.002	0.000	0.002	0.004	0.004	0				
<i>P. armatus</i> -BR(SC)	0.021	0.017	0.021	0.002	0.000	0.002	0.004	0.004	0.000	0			
<i>P. armatus</i> -BR(BA)	0.019	0.015	0.019	0.002	0.002	0.008	0.009	0.009	0.009	0.009	0		
<i>P. armatus</i> -BR(BA)	0.017	0.013	0.017	0.000	0.000	0.006	0.008	0.008	0.008	0.008	0.002	0	

of the resurrection of 2 previously separated genera of *Megalobrachium* Stimpson 1858 and *Porcellanopsis* Rathbun 1910, synonymized to the first by Haig (1956). Meanwhile, the proximity between some members of group I (“smooth” of Stillman and Reeb 2001) assigned as *Petrolisthes* and some members of the genus *Megalobrachium* evidenced in this work remains unclear. This result, based on a reduced molecular dataset for both genera, raises new questions about the phylogenetic relation of these genera and should be investigated in the future.

The largest *Petrolisthes* group (clade B, group II or “spiny” of Stillman and Reeb 2001) is characterized by species bearing teeth or spines on the anterior margin of the carpus and 1 or 2 posterodistal spines on the merus of pereopod 1. In addition, members of this group may share any or all the following morphological characters: epibranchial and supraorbital spines; a row of spines on the outer margin of the manus and on the anterior margin of the pereopods; and spines on the frontal, orbital, and lateral margins of the carapace. Most species of this group show transverse piliferous striations on the carapace and were described as a “*P. galathinus* group” and “*P. lamarcki* group” by Ortmann (1897). Posteriorly, the results of Hiller et al. (2006) based on molecular and geometric morphological data, strongly supported the validity of 6 nominal species comprising the *P. galathinus* complex (i.e., *P. galathinus*, *P. caribensis*, *P. columbiensis*, *P. sanmartini*, *P. bolivariensis*, and the more-distantly related, *P. rosariensis*). The results of our study agree with those conclusions, of *P. rosariensis* being separate from the main *P. galathinus* group and more-closely related to *P. glasselli*, a species that is also closely related to *P. galathinus*.

The *P. armatus* clade was shown to be a sister clade of the *P. robsonae* + *P. zaca* group: the first species was allied to *P. armatus* from which it may be distinguished morphologically by the presence of 2, instead of 3, spines on the carpus of the chelipeds, the reduced number of spines on the merus of the 1st 2 pairs of pereopods, and the distinct anterodistal spine on the carpus of the pereopods (Haig 1960). Rodríguez et al. (2006) found close relationships among *P. marginatus*, *P. politus*, and *P. armatus*. However, our analysis indicated a remote proximity among them, corroborating Haig’s conclusions based on morphological data.

Adults of *P. armatus* are known to show considerable morphological plasticity throughout

its geographical distribution range (Chace 1956, Haig 1960, Gore 1972). Some of these authors speculated that the variation seen in extreme examples of the Pacific and eastern Atlantic adults would, by itself, be sufficient to establish specific recognition between the members of the 2 regions. Considerable differences were also found when comparing larval morphology of Atlantic and Pacific specimens (Gore 1972). Nevertheless, our analysis showed that *P. armatus* formed a monophyletic clade, composed of specimens from different locations across its distributions along the Atlantic and Pacific coasts of the Americas. This result was corroborated by the morphological analysis, in which all variations observed were expected and insufficient to distinguish different morphological patterns. The present study did not include specimens from African populations, which should be the next step in clarifying phylogenetic relationships between African and Pacific-Atlantic populations.

In recent decapod taxonomy, some populations that were long regarded as geographically separated morphological variants were assigned to a full species rank based on molecular results (Sarver et al. 1998 2000, Fratini and Vannini 2002, Spivak and Schubart 2003, Schubart et al. 2005, Mantelatto et al. 2006 2009b). Additionally, some studies demonstrated intraspecific differences along latitudinal gradients in the reproductive biology of decapods (Clarke 1987, Wehrtmann and Kattner 1998, Lardies and Wehrtmann 2001), including some populations of *P. armatus* from Brazil and Costa Rica (Wehrtmann et al. 2011).

The reasons for minor latitudinal differences in genetic features of marine decapods, as seen in *P. armatus* (Fig. 2), are still being discussed (see references above). This phenomenon may be related to larval characteristics. *Petrolisthes armatus* is characterized by having a larval development, which consists of 2 zoeae and 1 megalopal stage with a duration of 1-2 mo (Gore 1970 1972, Brossi-Garcia and Moreira 1996); the megalopal stage spends part of its time in oceanic waters before colonizing near-shore habitats (Miranda and Mantelatto 2009). Thus, the possibility of mixing of *P. armatus* larvae/megalopae along the Atlantic gyre system is 1 hypothesis to explain the genetic similarities observed among populations from the western Atlantic and also from populations along the Pacific side of the Isthmus of Panama. Moreover, intertidal decapod species of *Sesarma*, *Petrolisthes*, and *Alpheus* show the smallest

values of transisthmian differentiation, which may be explained by a genetic connection between Pacific and Atlantic populations even when the only salt-water connection between the 2 sides consists of swamps (Lessios 2008). Such a scenario may further support our finding of no considerable genetic differences between eastern Pacific and western Atlantic populations of *P. armatus*, a situation already reported for other intertidal decapods (Lessios 2008).

In our dataset, the groups formed by *Pachycheles* and *Neopisosoma* were sister clades to the *Petrolisthes* group. Our analysis supported the validity of *Neopisosoma angustifrons* (Benedict 1901) being separate from the *Pachycheles* genus (Fig. 2), corroborating Rodríguez's et al. (2006) conclusions. Considering that the genus *Neopisosoma* is composed of 7 valid species (Haig 1960, Werding 1986), it is premature to consider those results as a definitive resolution for this group. Our continuing efforts are focused on adding representative taxa and combining molecular and morphological information on *Pachycheles* and *Neopisosoma* (Miranda and Mantelatto unpubl. data), which may contribute to clarifying the enigmatic status of these 2 genera. The present study showed that there is no clear separation among selected Porcellanidae genera from the American Atlantic and Pacific Oceans, as was assumed by previous studies (Stillman and Reeb 2001, Rodríguez et al. 2006). These assemblages seem to be a natural group that makes it impossible to separately study them in a phylogenetic context.

Our findings are in accordance with previous studies that discussed the great complexity within members of *Petrolisthes*, *Pachycheles*, and *Neopisosoma*. Pending the inclusion of additional genes and specimens from Africa and other areas of American waters in future analyses, the molecular data presented here confirm the monophyly of a well-defined *P. armatus* group in the genus.

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