

The *Petrolisthes galathinus* complex: Species boundaries based on color pattern, morphology and molecules, and evolutionary interrelationships between this complex and other Porcellanidae (Crustacea: Decapoda: Anomura)

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Abstract

While the amphi-American porcellanid crab *Petrolisthes galathinus* has been traditionally viewed as a highly variable species containing several different color forms, we consider it to be a complex of at least 6 morphologically similar species with similar ecological requirements, but diagnosable through coloration. Here we surveyed sequence variation of the mitochondrial 16S rRNA gene, compared the morphology of adults and of the first larval stage (Zoea I), and explored shape variation of the sternal plate using geometric morphometric methods, to investigate boundaries among the species in the complex, and to confirm the validity of color and color pattern for distinguishing them. Sequences and larval morphological characters of other porcellanids were included to investigate the correspondence between genetic divergence and morphology of adults and larvae. The molecular and morphometric results support the validity of the species in the complex, and of color pattern for their distinction. The close relationship between the complex and the putative ancestral porcellanid *Parapetrolisthes tortugensis* was indicated by the molecular and larval-morphology results. The adult morphology of this species is interpreted as a result of convergent evolution driven by a relatively rapid ecological adaptation to conditions in deeper waters. The nesting position in the phylogenetic trees of *Petrocheles australiensis* outside the Porcellanidae clade questions the monophyly of this family.

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1. Introduction

Porcellanidae Haworth, commonly known as porcelain crabs, is a diverse family of marine crab-like decapod crustaceans, with most of its species occurring in tropical regions. From the about 260 currently recognized species, classified in nearly 30 genera, approximately 110 occur in the Indo-West Pacific, 92 in the East Pacific, 48 in the West Atlantic, and 17 in the East Atlantic (Hiller et al., 2004;

Werdning et al., 2003). Although Porcellanidae is recognized as a well-defined taxon, accepted as a distinct family in the anomuran superfamily Galattheoidea together with the Galatheidae, Chirostylidae and Aegliidae, evolutionary relationships within the family are poorly known. The crab-like body form, more evident in porcellanids than in any other galatheid, makes these crabs an ideal group for evolutionary studies on carcinization processes in anomurans and brachyurans (true crabs). From an ecological perspective, Porcellanidae is an interesting taxon considering the preference of most species for shallow waters and a unique filter-feeding habit not present in any other galatheid family. Most species occupy hard-substrate habitats typically

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found on wave-exposed rocky shores and coral reefs, several others exhibit symbiotic associations with other marine invertebrates (Werding, 1983a), and a few ones are found in muddy burrows in mangrove swamps, and in estuarine waters (Werding and Hiller, 2004). Porcellanids can be considered a uniform taxon from an ontogenetic view as well. According to descriptions of larval development, these crabs usually hatch out as a zoea, passing through a second zoeal stage before metamorphosis to a megalopa. From a biogeographic perspective, Porcellanidae is an interesting taxon as a model to explore the influence of the Isthmus of Panama in driving speciation, as it counts with six nominal trans-isthmian species and a series of species pairs.

1.1. Taxonomy and distribution of *Petrolisthes* and the *Petrolisthes galathinus* complex

Petrolisthes Stimpson is the largest genus of the family comprising nearly 100 species worldwide. Although all species share a flattened body shape, they are morphologically highly diverse, and were divided by Ortmann (1897) in different groups according to morphological similarities. Alluding to Ortmann's divisions, Haig (1960) established five morphological lines into which most *Petrolisthes* species tend to fall. In the largest group Haig gathered Ortmann's "Gruppe des *P. galathinus*" and "Gruppe des *P. lamarcki*," which consist of forms characterized by teeth or spines on the chelipeds, carapace and walking legs. Another large group, containing the type species of the genus, *Petrolisthes violaceus*, consists of species in which these characters lack teeth or spines (Haig, 1960). We will refer to these groups as the *P. galathinus*–*P. lamarckii* group, containing the "armed-carpus" species, and the *P. violaceus* group, to which the "unarmed-carpus" species belong.

From a morphological perspective, three species, formerly included in *Petrolisthes*, and later transferred to two different genera, are of special interest. The Indo-West Pacific *Petrocheles* was created by Miers in 1876 as a subgenus of *Petrolisthes* to enclose *Petrocheles spinosus* (Miers, 1876) and *P. australiensis* (Miers, 1876). The Western Atlantic *Parapetrolisthes* was established by Haig (1962) to accommodate *Parapetrolisthes tortugensis*, originally described as *Petrolisthes tortugensis* by Glassell (1945). Both *Petrocheles* and *Parapetrolisthes* share characters with the *P. galathinus*–*P. lamarckii* group, but show other features absent in any other *Petrolisthes* species. Some authors (Baker, 1905; Miers, 1876) mentioned the strong resemblance between *Petrocheles* and the galatheid genus *Galathea* (family Galatheidae), and Glassell (1945) considered the peculiar pincer-shaped fingers of *P. tortugensis* as reminiscent of a number of forms among galatheids. Glassell (1945) considered his species to resemble both *Petrocheles* species. However, Haig (1960) considered *P. tortugensis* as "generically distinct from both *Petrolisthes* and *Petrocheles*," and therefore created this monotypic genus two years later. Regarding porcellanid ontogenetic features, Gore (1979) mentioned the apparent galatheid ancestry of

Petrocheles, as it undergoes more than two zoeal stages during development, what is typical of the Galatheidae. Within the context of the carcinization process in both galatheoid families, Glaessner (1960) suggested that porcellanids descended from galatheids. Some authors considered the two taxa to be sister families, as suggested by evidence from adult and spermatozoal morphology (Haig, 1960; Martin and Abele, 1986; McLaughlin and Lemaitre, 1997; Schram, 2001; Tudge, 1997) and genetic data (Morrison et al., 2002). Therefore, *Parapetrolisthes* and *Petrocheles* constitute key taxa for providing a unique insight into the evolutionary history of both families.

Stillman and Reeb (2001) adopted a first molecular approach to investigate evolutionary relationships within *Petrolisthes* by investigating sequence divergence of a fragment of the mitochondrial 16S rRNA gene among several eastern Pacific species. The molecular phylogenetic tree presented by these authors showed strong support for two main clades, referred to as "spiny" and "smooth." All species included in the spiny clade corresponded to the *P. galathinus*–*P. lamarckii* group. The smooth clade consisted of a heterogeneous set of species, including the species of the *P. violaceus* group, as well as different genera erected by Haig in 1960.

Species interrelationships within *Petrolisthes* still remain unclear, and as emphasized by Haig (1960, 1962), a revision of the entire genus on a worldwide basis is needed before any attempt of splitting it into different genera.

P. galathinus has been the subject of considerable taxonomic interest and conflict. It was originally described by Bosc (1802) as *Porcellana galathina* because of the transverse piliferous ridges covering carapace and extremities, resembling those of the galatheid genus *Galathea*. Much of the current taxonomic confusion can be traced to the original ambiguous description by Bosc, who failed to specify a type specimen and did not mention any type locality. Gibbes (1850) described *Porcellana sexspinosa* from Florida, USA, giving a detailed description of the species, though later he synonymized his species with Bosc's *P. galathina* (Gibbes, 1854). Since then, *P. galathinus* has been reported as a ubiquitous and highly variable species, showing a considerable color variation and a wide geographic distribution in the West Atlantic, from North Carolina, USA, to southern Brazil through the Gulf of Mexico and the Caribbean Sea, and in the eastern Pacific from El Salvador to Ecuador (Benedict, 1901; Gore, 1982; Hiller et al., 2004; Rickner, 1975; Williams, 1984). Werding (1977, 1982, 1983b) recognized the presence of different western Atlantic forms morphologically very similar to *P. galathinus*, but distinguishable through color and color pattern. All forms were originally found inhabiting the interstices formed by the coral *Porites porites* (Pallas) in the Rosario Archipelago, off the Colombian Caribbean coast. These findings led this author to describe *Petrolisthes rosariensis*, later reported from Cuba, Central America and tropical Brazil, *P. caribensis* with a current distribution from Florida, through the Antilles, to Pan-

ama and Colombia, and *P. columbiensis*, known only from the Rosario Archipelago and Cuba (see Werding, 1982, 1983a,b). Werding (1983b) referred to these species as the *P. galathinus* complex. Recent collections of Porcellanidae on the Caribbean coast of Colombia revealed two additional color forms described as *P. sanmartini* Werding and Hiller, 2002, so far not reported anywhere else, and *P. bolivarensis* Werding and Kraus, 2002, later found in Florida and Venezuela by the authors. A variety of new color forms morphologically matching *P. galathinus*, and an additional one, morphologically consistent with *P. caribensis* were also identified from this material. The coloration of the *P. galathinus* specimens recently collected in the tropical eastern Pacific differed from all color varieties in the western Atlantic.

Color documentation of the *P. galathinus* complex can be viewed at <http://www.uni-giessen.de/porcellanidae/index.htm#pictures>.

Currently, Werding's species complex consists of six described species, including *P. galathinus*. An interesting aspect of this complex is the different body size of the species, some of them (e.g. *P. bolivarensis*) reaching sizes that double or triplicate the maximum sizes, so far recorded, of relatively small species (e.g. *P. rosariensis*). All species show the same type of body striation as *P. galathinus*, and with one exception, each species can be morphologically distinguished from *P. galathinus* and from each other by a unique combination of subtle discrete differences (Table 1 and Fig. 1). *P. bolivarensis* shares identical states for these characters with *P. galathinus*, but nevertheless, the former species has a more robust appearance and its typical coloration clearly distinguishes it from all *P. galathinus* color forms, particularly by a conspicuous orange spot on the junction of the chelipeds' fingers. All species and color forms comprising the complex, except *P. caribensis*, display dark-red to purple lines and dots along the transversal striations covering the carapace and part of the extremities. *P. caribensis* has a uniformly carmine red carapace with a characteristic white cross in the frontal region. In the new color variation matching *P. caribensis* (see above), and designated as "Blue" because of its general pale blue appearance, the white cross on the front is absent, and the carapace displays red transverse ridges. Bright colors and patterns in the distal segments of the walking legs (carpus, propodus and dactylus) characterize each species and color form in the complex, varying from

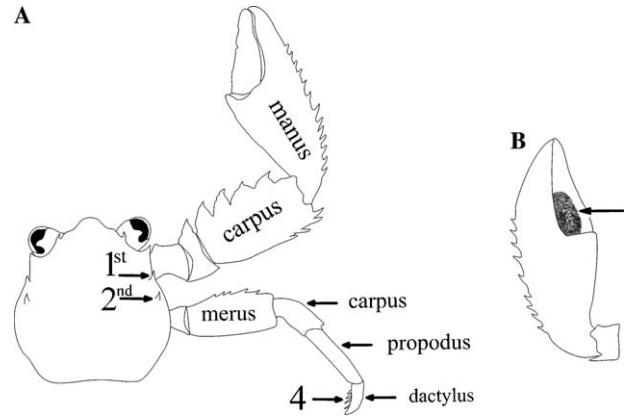


Fig. 1. Schematic representation of the characters used to distinguish the species of the *P. galathinus* complex. (A) Carapace showing both epibranchial spines (the first is always present), and dactylus of walking leg with 4 spines (3 or 5 spines are alternative states). Only one cheliped and one walking leg are shown, for which the main segments are designated. (B) Ventral view of manus of cheliped, with arrow pointing at pubescence.

purple with transverse white to yellow lines, yellow with longitudinal red stripes, to an intense orange lacking a striped pattern. The third maxillipeds are longitudinally striped with red and yellow or blue lines and the distal segments are conspicuously colored. The three different color forms matching *P. galathinus* were designated according to the most characteristic aspects of their coloration as follows: (1) "White Teeth," because the teeth of the chelipeds' carpus are mostly white-marked; (2) "Spots," because more dots than stripes demark the transverse striations of the crab's body; (3) "Stripes," because more stripes than dots delineate the transverse striations of the body. A series of specimens, particularly juveniles, show a combination of patterns 2 and 3, making their characterization difficult.

In the molecular phylogenetic survey of eastern Pacific *Petrolisthes* species, Stillman and Reeb (2001) included seven specimens of the complex, questioning the applicability of the coloration patterns given by Werding (1983b) for species identification, and highlighting the need for further taxonomic work on *P. galathinus* to better resolve species relationships.

Disentangling assemblages of morphologically similar but reproductively incompatible taxa demand the use of alternative sources of information. In several crustacean groups, larval characters proved to have a wider scope on

Table 1
Discrete morphological characters that distinguish the species of the *P. galathinus* complex

Species	Presence of second epibranchial spine	Nr. of spines on dactylus of walking legs	Presence of pubescence in gape of fingers of chelipeds
<i>Petrolisthes galathinus</i>	No	3	Yes
<i>Petrolisthes bolivarensis</i>	No	3	Yes
<i>Petrolisthes caribensis</i>	No	4	Yes
<i>Petrolisthes columbiensis</i>	Yes	4	Yes
<i>Petrolisthes rosariensis</i>	Yes	4	No
<i>Petrolisthes sanmartini</i>	No	5	Yes

the determination of natural classifications than some of the adult characters that define the present systematics do (Gurney, 1924; Wear, 1965). Within the Porcellanidae, two main species assemblages were established by Lebour (1943) based on characters from both zoeal stages. One of these groups was designated as the “*Petrolisthes* group.” Subsequent authors included some species in this group and declared others not to fit in any of Lebour’s assemblages (see Gore, 1972; Osawa, 1995). Since larval characters have a unique morphology, with a mainly independent development from adult characters, they constitute a promising and alternative source of information for inferring evolutionary relationships within Porcellanidae, and for aiding in the unraveling of species complexes.

Another interesting approach for disentangling complexes of species lacking discrete characters for their distinction is the study of shape variation of different corporal structures. Geometric morphometric procedures based on homologous structures (Bookstein et al., 1985) allow the analysis of shape differences in different species, and enable the application of multivariate statistical methods. In their study of shape variation among several Talpidae species, Rohlf et al. (1996) found small but significant shape differences that characterized each species, using the methods of generalized least-squares superimposition (Rohlf and Slice, 1990) and relative-warp analysis (Bookstein, 1991). These results allowed conducting a canonical variate analysis, and aligning the species in a hierarchical tree showing the shape similarity. This approach was adopted in a preliminary examination of the *P. galathinus* complex, proving to be useful for species distinction.

Coloration has proved to be of systematic importance among several species of decapod crustaceans (Bruce, 1975; Knowlton, 1986; Knowlton and Mills, 1992; Macpherson, 1994; Macpherson and Machordom, 2001; Sarver et al., 1998). Nevertheless, their use is limited considering that most material available for morphological comparison is preserved with the consequent color-fading, and that description of coloration of most crustacean species are incomplete or lacking.

Molecular data have contributed most significantly in areas where morphological data are inconclusive, deficient,

non-existent or poorly analyzed, and may be useful in identifying non-molecular characters that are phylogenetically informative (Avise, 1994; Patterson et al., 1993). The 16S rRNA and other mitochondrial genes have proved to be useful for molecular phylogenetic studies within the Crustacea at different taxonomic levels (Wetzer, 2001). This gene, in combination with other molecular markers and/or non-molecular data, have proven useful in giving taxonomic resolution within species complexes in a number of decapod crustaceans (Geller et al., 1997; Kitaura et al., 2002; Maggioni et al., 2001; Mathews et al., 2002; Sarver et al., 1998).

Here, we examine species boundaries among the six species comprising the *P. galathinus* species complex, and test the validity of coloration as an important character to distinguish species, by surveying sequence variation of the mitochondrial 16S rRNA gene, discrete adult and larval morphological characters, and shape variation of the sternal plate of the crabs (Fig. 2A). Additional *Petrolisthes* species, representing the *P. galathinus*–*P. lamarckii* group and the *P. violaceus* group, were included in the molecular analysis to locate the species of the *P. galathinus* complex within a phylogenetic frame. Based on results from preliminary molecular analyses (results not shown, see below) we included *Parapetrolisthes tortugensis*, *Petrocheles australiensis* and *Galathea squamifera* (family Galatheidae) in the final molecular data set, using the hermit crab *Pagurus longicarpus* (Anomura: Paguridae) as outgroup to both families. The resulting phylogeny was used to check the monophyly of Porcellanidae, to address the taxonomic status of *Parapetrolisthes* and *Petrocheles*, and to test the correspondence among evolutionary lines within *Petrolisthes*, as suggested by the morphology of adults and larvae.

2. Materials and methods

2.1. Preliminary analyses and taxon sampling

Preliminary analyses consisting of Bayesian inference on a multiple alignment of 95 mitochondrial 16S rRNA sequences comprising 12 different porcellanid genera (including *Parapetrolisthes*, *Petrolisthes*, and *Petrocheles*), and using the galatheid *G. squamifera* as outgroup, were conducted primarily

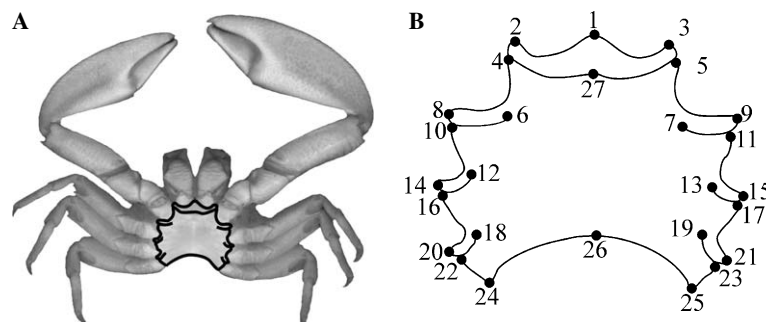


Fig. 2. Sternal plate selected for the geometric morphometric analysis (abdomen removed for complete view). (A) Ventral view of a porcellanid specimen showing the location of the sternal plate (marked with black line). (B) Schematic representation of the sternal plate with the 27 landmarks of types I and II.

to examine the nesting position of *Petrocheles* and *Parapetrolisthes* within Porcellanidae (results not shown). This alignment was generated using the software SOAP v.1.1 (Löytynoja and Milinkovitch, 2001) varying the gap opening penalty between 5 and 25 in steps of 2, and the gap extension penalty between 0.05 and 10.5 in steps of 0.5. Gaps were treated as missing data, as their inclusion in some regions of the alignment proved to be ambiguous under all gap inclusion/extension penalties tested. In the resulting trees *Parapetrolisthes tortugensis* unexpectedly nested within the *Petrolisthes galathinus* complex, while *Petrocheles australiensis* was always placed at a basal position. These results prompted us to include *G. squamifera* in the ingroup and to use an outgroup for Porcellanidae and Galatheidae. We opted for a taxon belonging to a different anomuran superfamily, the hermit crab *Pagurus longicarpus*, for which the complete 16S rRNA sequence is available in GenBank (Accession No. AF150756). This procedure resulted in the grouping of *Petrocheles australiensis* and *G. squamifera* in a clade located at the base of the porcellanid phylogeny.

From the data set used in the preliminary analyses, we concluded that the validity of such a global alignment is questionable considering the significant variation in the 16S rRNA gene among divergent species, as revealed by several unstable sites resulting from alignment under different gap inclusion/extension penalties. Therefore, all taxa not relevant for this survey were excluded from the final molecular data set, allowing us to perform a more careful alignment of the sequences in question.

Taxa included in the final data set and their corresponding sampling localities are listed in Table 2. In total, 97 specimens representing the six nominal species of the *P. galathinus* complex, other 15 *Petrolisthes* species, *Parapetrolisthes*, *Petrocheles*, and the galatheid *G. squamifera*, were sampled for this study. *Petrocheles australiensis* specimens were provided by the Museum Victoria, Melbourne, Australia, for DNA isolation. The sequence of the hermit crab *Pagurus longicarpus*, used in the preliminary analyses (see above) was used as outgroup to Porcellanidae and Galatheidae. Color documentation of the *P. galathinus* complex was carried out prior to specimen preservation. For two specimens, identified as *P. galathinus*, and collected in Brazil and Guyana in 1976, coloration could not be documented because colors faded due to their long time preservation in alcohol.

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from the chelipeds, walking legs and gills, using the Qiagen DNeasy® Kit (Qiagen GmbH, Hilden, Germany), following the protocol for animal tissues. A 520 bp region of the mitochondrial 16S rRNA gene was amplified using the primers 16sar-L and 16sbr-H (Palumbi et al., 1991). Double-stranded amplifications were performed in 25- μ l volume reactions containing 10 \times Taq buffer, Taq polymerase, 1.3 mM dNTPs (QBiogene, Inc., Heidelberg, Germany), each primer at 20 μ M, and ddH₂O.

DNA volumes varied from 0.3 to 2 μ l. PCR conditions consisted of an initial denaturation at 96 °C for 3 min, followed by 40 cycles of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min. A final extension step at 72 °C for 5 min followed the last cycle. PCR products were sequenced in both directions at the Service de Systématique Moléculaire, Muséum National d'Histoire Naturelle, Paris. Sequences were submitted to the GenBank data base under Accession Nos. DQ444866–DQ444962.

2.3. Sequence alignment and phylogenetic analysis

The BioEdit Sequence Alignment Editor (Hall, 1999) was used for sequence edition and identification of identical haplotypes. For homology maximization among sequences of members of the *P. galathinus* complex, they were aligned separately, and subsets of sequences, selected according to increased levels of divergence, were added to this alignment using the profile alignment implemented in CLUSTAL X 1.81 (Jeanmougin et al., 1998). Parsimony (MP) analyses were performed with PAUP* version 4.0b8 (Swofford, 2001), consisting of heuristic searches with 50 random-addition replicates and the TBR branch-swapping option. Gaps were treated as a fifth character state. Nodal support was estimated using bootstrap resampling with 1000 replicates (Felsenstein, 1985). Reliability of clades in the resulting MP trees was assessed by calculating decay indices (Bremer, 1988) using the programs TreeRot.v2b (Sorenson, 1999) and PAUP*.

Hierarchical likelihood ratio tests (hLRTs), as implemented in the program Modeltest 3.7 (Posada and Crandall, 1998), were conducted to select the model of nucleotide substitution that best fits the data. The estimated parameter values were used to conduct a full likelihood analysis. This procedure was conducted with PAUP*. The model of evolution determined by the hLRTs to better fit the data set was TVM + I + G ($-\ln L = 4815$).

Information of sequence evolution obtained by Modeltest was incorporated into the Bayesian analysis, performed with MrBayes3 (Ronquist and Huelsenbeck, 2003). The GTR model, with 6 free parameters and 2 extra free parameters, one for proportion of invariant sites, and one for the gamma distribution, was used. Chains were run for 2,000,000 generations and the first 200,000 were discarded for burnin. The remaining trees were used to estimate posterior probabilities to determine clade credibility.

Maximum likelihood (ML) and uncorrected pairwise distances (p) for all possible pairs of taxa were estimated using PAUP*, and were contrasted in a saturation plot. ML distances were computed with the substitution model used to obtain the ML tree.

The complete data set and one consisting of the species complex only were subjected to a test of rate constancy using a likelihood-ratio test (Felsenstein, 1988). PAUP* was used to obtain likelihood scores for the best fitting model without enforcing a molecular clock, and scores for the identical model with a molecular clock enforced.

Table 2
Specimens sequenced for this study, sampling localities and collection data (taxa listed in alphabetical order)

Species	<i>n</i>	Sampling localities and collection data
<i>Galathea squamifera</i>	1	EA, France, Saint Maló. Intertidal. Coll. Sept. 2000. R. Nr. IV-1.
<i>Parapetrolisthes tortugensis</i> ^a	4	WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 10–12 m. Coll. Apr. 2001. R. Nr. X-27 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 10–12 m. Coll. Apr. 2001. R. Nr. X-28 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 10–12 m. Coll. Apr. 2001. R. Nr. X-67 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 10–12 m. Coll. July 2001. R. Nr. B-6A
<i>Petrocheles australiensis</i>	1	IWP, Australia, Victoria, Twin Reefs, Cape Paterson. Museum Victoria, Melbourne—J15588
<i>Petrolisthes agassizii</i>	1	EP, Colombia, Gorgona Island. Intertidal. Mar. 2000. R. Nr. II-38
<i>Petrolisthes amoenus</i>	1	WA, Southern Caribbean, Venezuela, Cubagua Island. 1.5 m. Coll. Apr. 2004. R. Nr. q-38
<i>Petrolisthes armatus</i> ^a	2	WA, USA, Florida, Fort Pierce, Harbor Branch. Intertidal. Coll. June 2001. R. Nr. XIII-5A EP, Ecuador, Salinas. Intertidal. Coll. Jan. 2002. R. Nr. D-45
<i>Petrolisthes bolivarensis</i> ^a	7	WA, USA, Florida, Key Biscayne. 1 m. Coll. June 2001. R. Nr. XIII-21 WA, Southern Caribbean, Venezuela, Cubagua Island. 1.5 m. Coll. June 2002. R. Nr. J-5A WA, Southern Caribbean, Colombia, San Bernardo Islands. 1.5 m. Coll. Feb. 2002. R. Nr. G-12A WA, USA, Florida, Key Biscayne. 1 m. Coll. June 2001. R. Nr. XIII-31 WA, USA, Florida, Key Biscayne. 1 m. Coll. June 2001. R. Nr. XIII-20 WA, Southern Caribbean, Colombia, San Bernardo Islands. 1.5 m. Coll. Feb. 2000. R. Nr. I-168 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 2 m. Coll. Feb. 2002. R. Nr. F-15A
<i>Petrolisthes caribensis</i> ^a	7	WA, USA, Florida, Key Biscayne. 1 m. Coll. June 2001. R. Nr. XIII-14 WA, USA, Florida, Key Biscayne. 1 m. Coll. May 2004. R. Nr. XIII-34 WA, Southern Caribbean, Colombia, San Bernardo Islands. 1.5 m. Coll. Feb. 2000. R. Nr. I-145 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 2 m. Coll. July 2001. R. Nr. B-9C WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 1.5 m. Coll. July 2001. R. Nr. C-8A WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1 m. Coll. July 2001. R. Nr. B-9B WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1 m. Coll. July 2001. R. Nr. B-9Agest.
<i>Petrolisthes caribensis</i> “Blue”	9	WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. Feb. 2002. R. Nr. F-7B WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 2 m. Coll. July 2001. R. Nr. C-9A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 1.5 m. Coll. Feb. 2002. R. Nr. G-7A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 2 m. Coll. Feb. 2002. R. Nr. G-8B WA, Southern Caribbean, Colombia, San Bernardo Islands. 2 m. Coll. Feb. 2002. R. Nr. G-25A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, Punta Piedra. 1.5 m. Coll. Feb. 2002. R. Nr. G-4 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. July 2001. R. Nr. B-16A WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. July 2001. R. Nr. B-16B WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. Feb. 2002. R. Nr. F-7A
<i>Petrolisthes columbiensis</i> ^a	5	WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 2 m. Coll. July 2001. R. Nr. B-10A WA, Southern Caribbean, Colombia, San Bernardo Islands. 2 m. Coll. Feb. 2000. R. Nr. I-170 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 2 m. Coll. July 2001. R. Nr. B-10B WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 2 m. Coll. Feb. 2002. R. Nr. F-1A WA, Southern Caribbean, Colombia, San Bernardo Islands. Coll. 2 m. Coll. Feb. 2000. R. Nr. I-150

Table 2 (continued)

Species	<i>n</i>	Sampling localities and collection data
<i>Petrolisthes edwardsii</i> ^a	1	EP, Ecuador, Salinas. 1 m. Coll. Jan. 2002. R. Nr. D-28A
<i>Petrolisthes galathinus</i> pres.	2	WA, French Guyana, Iles du Salut. 5 m. Coll. May 1972. R. Nr. VIII-13A
<i>Petrolisthes galathinus</i> pres.		WA, southern Brazil, Pará. 5 m. Coll. June 1972. R. Nr. VIII-11A
<i>Petrolisthes galathinus</i> “White Teeth”	9	WA, USA, Florida, Key Biscayne. Intertidal. Coll. June 2001. R. Nr. XIII-3A WA, USA, Florida, Key Biscayne. Intertidal. Coll. June 2001. R. Nr. XIII-3B WA, Southern Caribbean, Venezuela, Cubagua Island. 1.5 m. Coll. Apr. 2004. R. Nr. q-22A WA, USA, Florida, Key Biscayne. Intertidal. Coll. June 2001. R. Nr. XIII-2 WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. July 2001. R. Nr. B-18A WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. July 2001. R. Nr. B-18B WA, Southern Caribbean, Venezuela, Cubagua Island. 1.5 m. Coll. June 2002. R. Nr. J-7A WA, Southern Caribbean, Venezuela, Cubagua Island. 1.5 m. Coll. June 2002. R. Nr. J-8A WA, Southern Caribbean, Venezuela, Cubagua Island. 1.5 m. Coll. June 2002. R. Nr. J-8C
<i>Petrolisthes galathinus</i> “Spots” ^a	10	WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. Feb. 2002. R. Nr. F-11A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 2 m. Coll. Feb. 2002. R. Nr. G-6A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, Punta Piedra. 1.5 m. Coll. Feb. 2002. R. Nr. G-5A WA, Southern Caribbean, Colombia, Santa Marta. 2 m. Coll. Sep. 2002. R. Nr. K-4 WA, Southern Caribbean, Colombia, Santa Marta. 2 m. Coll. Sep. 2002. R. Nr. K-5 WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Sep. 2002. R. Nr. i-9A WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Sep. 2002. R. Nr. i-9B WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Sep. 2002. R. Nr. i-9C WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Feb. 2000. R. Nr. I-104 WA, Southern Caribbean, Colombia, Gulf of Urabá, Bay of Triganá. 2 m. Coll. Mar. 2004. R. Nr. R-1
<i>Petrolisthes galathinus</i> “Stripes” ^a	6	WA, Colombian Caribbean, Gulf of Morrosquillo, Tolú. 3 m. Coll. Apr. 2001. R. Nr. X-9 WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 3 m. Coll. Feb. 2002. R. Nr. G-1A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, Cabrana Island. 2 m. Coll. Feb. 2002. R. Nr. G-3A WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, Cabrana Island. 2 m. Coll. Feb. 2002. R. Nr. G-3B WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Sep. 2002. R. Nr. i-8C WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Sep. 2002. R. Nr. i-8 WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Sep. 2002. R. Nr. i-8B
<i>Petrolisthes galathinus</i> EP ^a	6	EP, Ecuador, Salinas. 1.5 m. Coll. Jan. 2002. R. Nr. D-19 EP, Colombia, Malaga Bay. 3–4 m. Coll. Sep. 2004. R. Nr. U-29 EP, Ecuador, Salinas. 1.5 m. Coll. Jan. 2002. R. Nr. D-26B EP, Ecuador, Salinas. 2 m. Coll. Jan. 2002. R. Nr. D-33 EP, Ecuador, Salinas. 1.5 m. Coll. Jan. 2002. R. Nr. D-26A EP, Colombia, Malaga Bay. 3–4 m. Coll. Sep. 2004. R. Nr. U-28A
<i>Petrolisthes glasselli</i>	1	EP, Colombia, Malpelo Island. 6 m. Coll. Sep. 2002. R. Nr. L-6A
<i>Petrolisthes haigae</i> ^a	1	EP, Ecuador, Salinas. 3 m. Coll. Jan. 2002. R. Nr. D-23 ^a
<i>Petrolisthes hirtispinosus</i>	1	EP, Mexico, Baja California, Bahía de los Angeles, Isla Angel de la Guarda. Intertidal. Coll. R. Wetzter, 12 March 2001. R. Nr. t-8
<i>Petrolisthes marginatus</i> ^a	2	WA, Southern Caribbean, Colombia, Santa Marta. 0.5 m. Coll. Jan. 2002. R. Nr. E-9A EA, Cape Verde. Coll. F. Lorenz, Apr. 2003. R. Nr. IX-15
<i>Petrolisthes monodi</i>	1	EA, Cape Verde. Coll. P. Wirtz, Oct. 2003. R. Nr. IX-16
<i>Petrolisthes quadratus</i>	1	WA, Southern Caribbean, Colombia, Santa Marta. Intertidal. Coll. Feb. 2000. R. Nr. X-31
<i>Petrolisthes robsonae</i>	1	EP, Panama, Darién, mouth of Tuira River. Intertidal. Coll. 1975. R. Nr. VIII-5A
<i>Petrolisthes rosariensis</i> ^a	6	WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 3 m. Coll. July 2001. R. Nr. C-10A WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 2 m. Coll. Feb. 2002. R. Nr. F-16A

(continued on next page)

Table 2 (continued)

Species	<i>n</i>	Sampling localities and collection data
		WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 1.5 m. Coll. Feb. 2002. R. Nr. F-16B
		WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. Coll. 2 m. Coll. Feb. 2002. R. Nr. F-4 A
		WA, Southern Caribbean, Colombia, Santa Marta. 1.5 m. Coll. Feb. 2000. R. Nr. I-133
		WA, Southern Caribbean, Colombia, Santa Marta. 2 m. Coll. Jan. 2002. R. Nr. E-16
<i>Petrolisthes sanfelipensis</i>	1	EP, Mexico, Baja California, Bahía de los Angeles, Isla Angel de la Guarda. Intertidal. Coll. R. Wetzler, 12 Mar. 2001. R. Nr. VIII-66
<i>Petrolisthes sanmartini</i>	5	WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 3 m. Coll. July 2001. R. Nr. B-15A
		WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. Coll. 4 m. Coll. Feb. 2002. R. Nr. F-3B
		WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 6 m. Coll. Apr. 2001. R. Nr. X-49
		WA, Southern Caribbean, Colombia, Rosario Archipelago, San Martín de Pajarales Island. 5 m. Coll. Apr. 2001. R. Nr. X-47
		WA, Southern Caribbean, Colombia, Gulf of Morrosquillo, near Punta San Bernardo. 3 m. Coll. July 2001. R. Nr. C-11
<i>Petrolisthes tonsorius</i> ^a	2	EP, Colombia, Cabo Corrientes. Intertidal. Coll. Sep. 2003. R. Nr. O-42A
		WA, Southern Caribbean, Venezuela, Cubagua Island. Intertidal. Coll. Apr. 2004. R. Nr. q-30
<i>Petrolisthes tridentatus</i> ^a	1	WA, Southern Caribbean, Venezuela, Cubagua Island. Intertidal. Coll. Apr. 2004. R. Nr. q-31
<i>Petrolisthes zaca</i>	1	EP, Colombia, Malaga Bay. Intertidal. Coll. Sep. 2004. R. Nr. U-40A

All material was collected by the authors unless specified; pres = preserved; EA = eastern Atlantic; WA = western Atlantic; EP = eastern Pacific; IWP = Indo-West Pacific; R. Nr. = personal reference number.

^a Species for which ovigerous females were collected for larval culture. See Section 2.

Differences in likelihood were contrasted against the χ^2 distribution for the requisite degrees of freedom.

The program MacClade (Maddison and Maddison, 2001) was used to examine type and distribution of substitutions supporting clades in the resulting phylogenetic trees.

2.4. Larval morphology

Ovigerous females of the species indicated in Table 2 by the superscript letter ‘a’ were collected on the Colombian Caribbean coast and at La Libertad, Ecuador, for subsequent larval culture. From the species of the *P. galathinus* complex only *P. sanmartini* could not be sampled, because no females carrying eggs were found during our collection efforts. Larval culture, preservation and dissection for observation were carried out following Kraus et al. (2004).

2.5. Sternal morphology

Preliminary analyses of shape variation of different corporal structures of several porcellanid species resulted in the selection of the sternal plate (Fig. 2A). In this structure several landmarks, i.e., discrete, homologous anatomical loci, were identified. All landmarks lie within the same plane, and can be found repeatedly and reliably, both conditions matching ideal criteria for landmark selection (see Zelditch et al., 2004). The sternal plate is relatively flat, what is adequate for an easy digitalization (see below) and a reliable landmark placement. Other structures like the

carapace and chelipeds, which, like the sternal plate, may also vary continuously among species, proved not to be convenient for these procedures, as they are not flat, making their digitalization difficult and landmark placement ambiguous. Furthermore, in these structures the possible landmarks lie in different planes and their homology between species is not always clear. The sternal plate proved to be a region that favors the use of the geometric morphometric method, not only due to the high stability compared to other skeletal elements, but also because of the variety of protrusions and channels found on this structure, which bring forth a multitude of high-grade Type I landmarks (see Bookstein, 1991). High-resolution digitized images of the sternal plate of a total of 295 individuals, representing the six species of the *P. galathinus* complex, *P. tortugensis* and the eastern Pacific *P. glasselli*, were gained by scanning the crabs using a modified fluid immersion (Ethanol 70%) flat bed scanner (Epson Perfection 4870[®]) with an optical resolution of 9600 dpi and a color depth of 48 bit. Specimens of the *P. galathinus* “Stripes” and eastern Pacific color forms, and of the *P. caribensis* “Blue” variant were included as well. In total, 27 landmarks of types I and II (Fig. 2B) were placed on each image using the tpsDig 1.40 software (Rohlf, 2003), which were then saved as Cartesian coordinates. To create a single file containing all images and landmark data, the tpsUtil 1.22 program (Rohlf, 2003) was used. At the beginning of the basic geometric morphometric analysis, the data file was opened using the software tpsRelW 1.39 (Rohlf, 2004). The type II landmarks (also known as pseudolandmarks) 26 and 27, as

well as the type I/II landmarks 24 and 25, were specified as sliding points by utilizing slider files according to Rohlf (2003). The next step consisted of the computation of the consensus configuration via the generalized orthogonal least-squares Procrustes procedure, based on complex regressions (Rohlf and Slice, 1990). This generalized orthogonal least-squares Procrustes average configuration is used as the reference configuration to which each dataset is compared. The settings for this computation were as follows: $\alpha=0$, no PCA align reference, complement uniform component, chord minimum BE slide method, five slide maximum iterations, scale alignment=1, and orthogonal projection. From the bending energy matrix, the principal warps were computed. Through projecting each specimen on these basic deformations, the partial warps could be gained and the matrix of partial warp scores, the weight matrix, was acquired. Since α was chosen to be zero the reckoning of the relative warps, that summarize the variations among the included individuals, is equal to a principal component analysis of the covariance matrix of the partial warp scores (Rohlf, 2004).

The software Microsoft Excel 2002[®] and Statistica 6.0[®] were used for the statistical analysis of the weight matrix. Univariate tests of significance were accomplished by dint of the *f*-test. Multivariate significance tests included Wilk's lambda, Pillai's trace, Hotelling–Lawley's trace analysis, and Roy's largest root test. For the calculations of Mahalanobis distances, a discriminant analysis based on the coordinate matrix was conducted. Finally, a number of agglomerative cluster analyses and k-means cluster analyses, with 1 – Person-*r* as distance measurement and the UPGMA fusion standard, concluded the examination of the datasets.

3. Results

3.1. Molecular data

3.1.1. Sequence properties and variability

The length of the 16S rRNA partial sequences of the 97 specimens included in the molecular data set ranged between 515 and 520 bp. Identical haplotypes were identified (Table 3) and excluded from the data set prior to subjecting this to phylogenetic analyses. Different haplotypes for each species or color form were designated as in Table 3. No individuals of the *P. galathinus* complex, corresponding to different morphospecies or color forms, shared identical haplotypes. More than one haplotype was identified for each species in the complex.

From a total of 97 sequences obtained for this survey 47 were identified as redundant haplotypes, and were excluded from the data set. The final data set subjected to phylogenetic analyses contained 51 sequences (Table 3) representing 23 porcellanid species, all the color variants identified in the *P. galathinus* complex, the galatheid *G. squamifera* and the outgroup *Pagurus longicarpus*. The resulting profile alignment comprised 535 sites corresponding to nucleotide

positions 649 to 1166 of the *Pagurus longicarpus* 16S rRNA gene, which is 1303 bp long.

No significant differences in sequence base composition were found across all taxa including the outgroup, as revealed by the Chi-square homogeneity test implemented in PAUP* ($P=1.000$). Mean base frequencies were 0.359 for A, 0.110 for C, 0.190 for G and 0.341 for T (in an average number of 514.73 sites), which do not substantially vary if non-porcellanid sequences are excluded. A high proportion of A and T nucleotides (70%), as compared with C and G, is concordant with values reported for the mitochondrial genome of other arthropod taxa (Crease, 1999; Simon et al., 1994).

3.1.2. Phylogenetic analyses based on molecular data

Of the 535 aligned sites, 285 were variable, of which 228 were parsimony-informative.

The parsimony analysis yielded 20 most parsimonious trees. The strict consensus of these trees is shown in Fig. 3A. One ML tree resulted from the likelihood analysis under the TV+I+G model (Fig. 3B). The topology of this tree was virtually identical to the one of the tree obtained through Bayesian inference (not shown). The MP and ML tree topologies were fully compatible, showing agreement in position and species composition of most clades. None of the trees supports a monophyletic status for Porcellanidae, regarding the nesting position of *Petrocheles australiensis*, as this species seems to share a more recent common ancestor with the Galatheidae than with the rest of porcellanids. *P. australiensis* and *G. squamifera* appear as sister taxa with a relatively high bootstrap (BT) and posterior probability (PP) supports. Although the sister-taxon relationship probably results from incomplete galatheid taxon-sampling, the clade containing these two species is supported by eight transversions. A porcellanid taxon-sampling problem can be ruled out in this case because in the preliminary analyses (see above), in which several porcellanid taxa were included, *Petrocheles australiensis* consistently nested together with *G. squamifera*. The strong support for this clade, as well as the relatively well supported clade containing the rest of Porcellanidae, showing eight transversions and large BT (98), PP (99) and DEC (decay index: 11) values, suggests that the exclusion of *Petrocheles australiensis* from the porcellanid clade may be real. Within the Porcellanidae clade, two main groups showed a relatively high nodal support, each containing species corresponding to each of the two largest *Petrolisthes* morphological assemblages, the *P. galathinus*–*P. lamarckii* group (species with an armed-carpus) and the *P. violaceus* group (species with an unarmed-carpus). Although the clade containing the unarmed-carpus species is underrepresented with only three species, the eight transversions supporting this clade, as well as the three transversions supporting the clade with the armed-carpus species, suggest that the molecular divergence shows correspondence with the substantial morphological differences between these two species assemblages. Within the unarmed-carpus species clade, the two *P. tonso-*

Table 3
Number and sampling origin of all sequences included in the molecular data set (taxa listed in alphabetical order), and haplotypes identified for species and color morphs for which more than one individual was sequenced

Species	Nr. of individuals (in bold) sequenced and sampling origin	Nr. (in bold) and sampling origin of haplotypes	Sequence names in data set and Nr. of individuals (in parentheses) sharing a unique haplotype
<i>Galathea squamifera</i>	1 : East Atlantic		<i>Galathea</i> -EA
<i>Pagurus longicarpus</i> ^b	1 : West Atlantic		<i>Pagurus</i> -WA
<i>Parapetrolisthes tortugensis</i>	4 : South Caribbean	1 : South Caribbean	<i>Parapetrolisthes</i> -WA
<i>Petrocheles australiensis</i>	1 : Indo-West Pacific		<i>Petrocheles</i> -IWP
<i>Petrolisthes agassizii</i>	1 : East Pacific		<i>P. agassizii</i> -EP
<i>Petrolisthes amoenus</i>	1 : West Atlantic		<i>P. amoenus</i> -WA
<i>Petrolisthes armatus</i>	1 : Florida	1 : Florida	<i>P. armatus</i> -WA
	1 : East Pacific	1 : East Pacific	<i>P. armatus</i> -EP
<i>Petrolisthes bolivarensis</i>	3 : Florida	1 : Florida	<i>P. bolivarensis</i> -Fla
	4 : South Caribbean	1 : South Caribbean	<i>P. bolivarensis</i> -Car
		1 : Florida and South Caribbean	<i>P. bolivarensis</i> -FlaCar (5)
<i>Petrolisthes caribensis</i>	2 : Florida	2 : South Caribbean	<i>P. caribensis</i> -Car1 (2)
	5 : South Caribbean	1 : Florida and South Caribbean	<i>P. caribensis</i> -Car2
			<i>P. caribensis</i> -FlaCar (4)
<i>Petrolisthes caribensis</i> “Blue”	9 : South Caribbean	2 : South Caribbean	<i>P. caribensis</i> Blue-Car1
			<i>P. caribensis</i> Blue-Car2 (8)
<i>Petrolisthes columbiensis</i>	5 : South Caribbean	3 : South Caribbean	<i>P. columbiensis</i> -Car1
			<i>P. columbiensis</i> -Car2
			<i>P. columbiensis</i> -Car3 (3)
<i>Petrolisthes edwardsii</i>	1 : East Pacific		<i>P. edwardsii</i>
<i>Petrolisthes galathinus</i> preserved ^a	1 : French Guyana	1 : French Guyana	<i>P. galat</i> -Guyana
	1 : South Brazil	1 : South Brazil	<i>P. galat</i> -Brazil
<i>Petrolisthes galathinus</i> “White Teeth”	2 : Florida	2 : Florida	<i>P. galat</i> WhiteTeeth-Fla1
	7 : South Caribbean	1 : Florida and South Caribbean	<i>P. galat</i> WhiteTeeth-Fla2
			<i>P. galat</i> WhiteTeeth-FlaCar (7)
<i>Petrolisthes galathinus</i> “Spots”	10 : South Caribbean	1 : South Caribbean	<i>P. galat</i> Spot-Car
<i>Petrolisthes galathinus</i> “Stripes”	7 : South Caribbean	2 : South Caribbean	<i>P. galat</i> Stripe-Car1 (4)
			<i>P. galat</i> Stripe-Car2 (3)
<i>Petrolisthes galathinus</i> East Pacific	2 : Colombia	1 : Colombia	<i>P. galat</i> EP-Col
	4 : Ecuador	1 : Ecuador	<i>P. galat</i> EP-Ecu
		1 : Colombia and Ecuador	<i>P. galat</i> EP-ColEcu (4)
<i>Petrolisthes glasselli</i>	1 : East Pacific		<i>P. glasselli</i> -EP
<i>Petrolisthes haigae</i>	1 : East Pacific		<i>P. haigae</i> -EP
<i>Petrolisthes hirtispinosus</i>	1 : East Pacific		<i>P. hirtispinosus</i> -EP
<i>Petrolisthes marginatus</i>	1 : South Caribbean	1 : South Caribbean	<i>P. marginatus</i> -WA
	1 : East Atlantic	1 : East Atlantic	<i>P. marginatus</i> -EA
<i>Petrolisthes monodi</i>	1 : East Atlantic		<i>P. monodi</i> -EA
<i>Petrolisthes quadratus</i>	1 : South Caribbean		<i>P. quadratus</i> -WA
<i>Petrolisthes robsonae</i>	1 : East Pacific		<i>P. robsonae</i> -EP
<i>Petrolisthes rosariensis</i>	6 : South Caribbean	4 : South Caribbean	<i>P. rosariensis</i> -Car1
			<i>P. rosariensis</i> -Car2 (3)
			<i>P. rosariensis</i> -Car3
			<i>P. rosariensis</i> -Car4
<i>Petrolisthes sanfelipensis</i>	1 : East Pacific		<i>P. sanfelipensis</i> -EP
<i>Petrolisthes sanmartini</i>	5 : South Caribbean	3 : South Caribbean	<i>P. sanmartini</i> -Car1 (3)
			<i>P. sanmartini</i> -Car2
			<i>P. sanmartini</i> -Car3
<i>Petrolisthes tonsorius</i>	1 : South Caribbean	1 : South Caribbean	<i>P. tonsorius</i> -WA
	1 : East Pacific	1 : East Pacific	<i>P. tonsorius</i> -EP
<i>Petrolisthes tridentatus</i>	1 : South Caribbean		<i>P. tridentatus</i> -WA
<i>Petrolisthes zacae</i>	1 : East Pacific		<i>P. zacae</i> -EP

WA = western Atlantic; EP = eastern Pacific; EA = eastern Atlantic; IWP = Indo-West Pacific; Fla = Florida; Car = Caribbean; Col = Colombia; Ecu = Ecuador.

^a *Petrolisthes galathinus* specimens preserved in 70% alcohol, no color documented. See text.

^b *Pagurus longicarpus* sequence obtained from GenBank; see text.

rius sequences from both American coasts clustered with seven transversions. Two main clades within the cluster containing the armed-carpus species can be distinguished in both MP and ML trees, though with relatively small BS, PP

and DEC values. The first clade is subdivided into two relatively well supported groups, one containing the species of the *P. galathinus* complex, excluding *P. rosariensis*, and including *Parapetrolisthes tortugensis*, and the other one

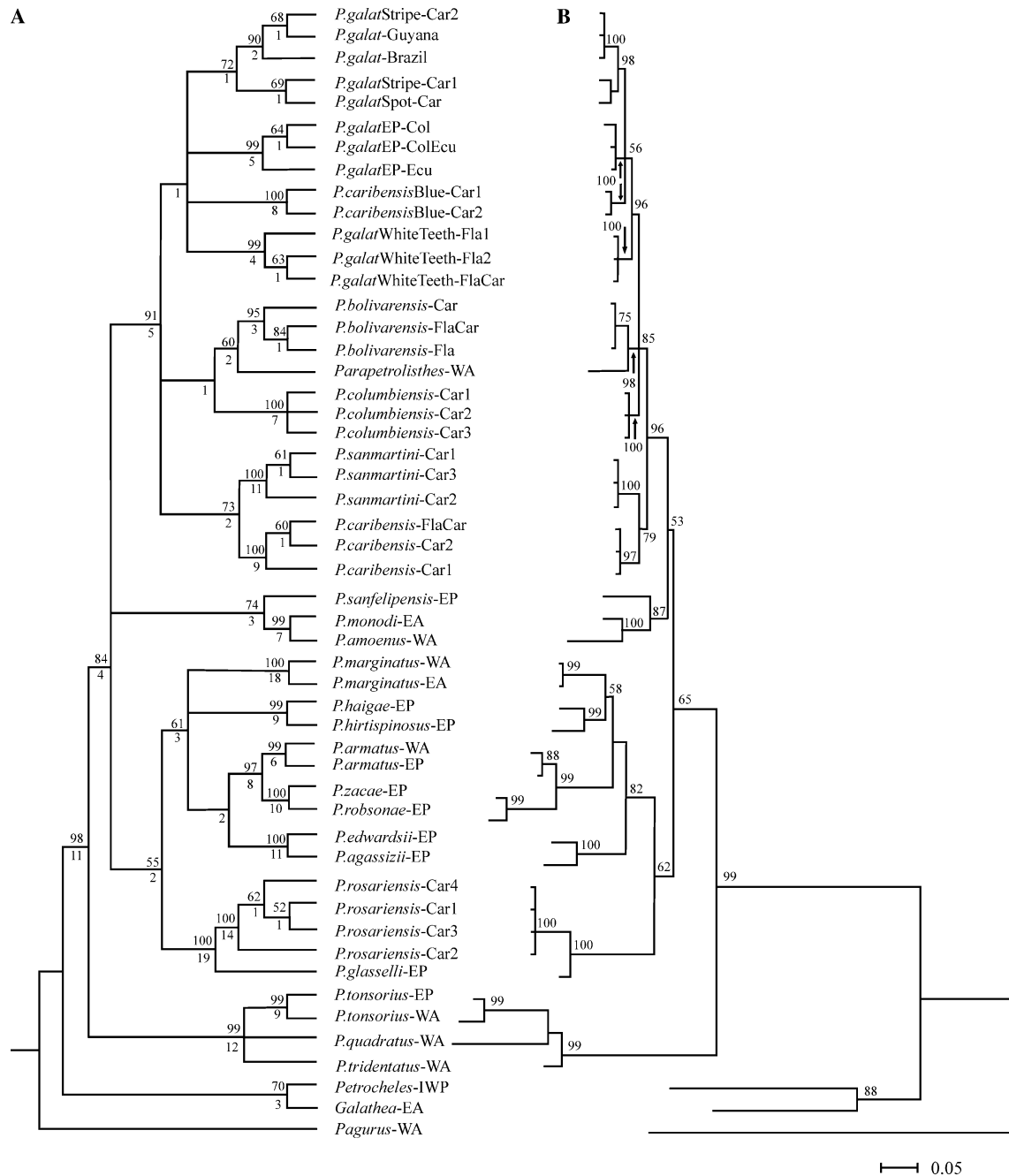


Fig. 3. Phylogenetic trees based on the data set corresponding to 51 sequences of a fragment of the mitochondrial 16S rRNA gene, representing 23 porcellanid species, one galatheid, and the hermit crab *Pagurus longicarpus* as outgroup. (A) Strict consensus of 20 most parsimonious trees ($L = 995$; $RI = 0.705$). Bootstrap values based on 1000 replicates shown above nodes (only values higher than 50% are shown). Decay values shown below nodes. (B) Maximum likelihood tree ($-\ln$ likelihood = 4805.3317; estimated proportion of invariable sites = 0.240; estimated value of gamma shape parameter = 0.385) obtained after a heuristic search (TBR swapping algorithm), using the parameters of the TV + I + G substitution model. Values on nodes correspond to posterior probabilities obtained from the Bayesian analysis (see text). See Table 3 for sequence-name and locality abbreviations. WA = western Atlantic; EP = eastern Pacific; IWP = Indo-West Pacific.

comprising the eastern Pacific *Petrolisthes sanfelipensis*, the western Atlantic *P. amoenus* and the eastern Atlantic *P. monodi*. All species of the *P. galathinus* complex (*P. rosariensis* excluded) cluster together in a clade supported by four transversions, though the resolution within this cluster is low. The placement of *P. tortugensis* within the *P. galathinus* complex clade, and particularly as sister taxon of *P.*

bolivarensis, appears most unusual from a morphological perspective. Somewhat unexpectedly the molecular data proved to be compelling in support of the placement of *Parapetrolisthes tortugensis* as sister taxon to *Petrolisthes bolivarensis* through a nonhomoplastic synapomorphic transversion supporting this clade. The placement of *P. tortugensis* in the same clade as the species of the complex, and

the exclusion of *P. rosariensis* from this group are consistent with larval features (see below), as well as with observations of a first DNA-sequence alignment of the species of the complex. Alignment of the *P. rosariensis* sequences to the complex resulted in the inclusion of seven gaps, while the *P. tortugensis* sequence could be aligned to the complex without gap inclusion. Strong support for the monophyly of each of the currently recognized species comprising the *P. galathinus* complex, except *P. galathinus*, was indicated in both trees. All sequences corresponding to different color variations morphologically matching *P. galathinus* were grouped in a clade together with the *P. caribensis* “Blue” variant. Although the *P. galathinus* clade was not supported by a large BS value in the MP tree, it showed high PP support in the ML tree and was supported by two transversions. The clade containing all sequences of the *P. galathinus* “White Teeth”-variant, as well as the one including all *P. galathinus* individuals from the eastern Pacific were strongly supported with large BS and PP values, though only the eastern Pacific clade was supported by two transversions, while the “White Teeth” clade was supported only by transitions. The sequences of the *P. caribensis* “Blue” variant clustered with four transversions. One of two haplotypes recovered for the “Stripes” color form (*P. galatStripe-Car1*) nested with the unique haplotype identified for the “Spots” variant. The second one (*P. galatStripe-Car2*) was included in the clade containing the two preserved specimens from Guyana and Brazil. The clade containing the “Stripes,” “Spots,” and the preserved specimens is supported by one transversion, and by a large PP value in the ML tree, though in the MP tree this clade is relatively poorly supported. These results suggest that the preserved specimens corresponded to *P. galathinus* and not to *P. bolivarensis*, which cannot be distinguished from the former species by discrete morphological characters. Further collections in the Gulf of Mexico, Florida and South Carolina, where *P. galathinus* has been reported to occur, are needed to clear the real geographic range of this species (see Section 4). In summary, every species and color variant comprising the complex segregate into strongly supported clades, except the *P. galathinus* “Stripes” and “Spots” variants, which group heterogeneously with each other and with the preserved specimens from Guyana and Brazil. These results are concordant with observations that often small individuals cannot be distinguished through coloration (see above), and may suggest that the “Stripe” and “Spot” variants represent a detectable color polymorphism of the species. The clade most closely related to the species of the complex is that containing the eastern Pacific *Petrolisthes sanfelipensis* and a clade consisting of the western Atlantic *P. amoenus* and the eastern Atlantic *P. monodi*, though the sister-taxon relationship between this clade and *P. sanfelipensis* is not strongly supported. The high support for the *P. amoenus*–*P. monodi* clade, as shown by relatively large BS (99), PP (100) and DEC (7) values, as well as three transversions, is concordant with their morphological resemblance, as these species are considered by the authors

to constitute an amphi-Atlantic species pair (see Section 4). The close relationship of these species to *P. sanfelipensis* is not congruent from a morphological perspective, probably resulting from incomplete taxon sampling.

The second clade, containing the rest of the armed-carpus *Petrolisthes* species, included *P. rosariensis*, which showed a strongly supported sister-taxon relationship with the eastern Pacific *P. glasselli*, evidenced by five transversions. Morphological characters like the presence of two epibranchial spines and four spines on the inner margin of the walking-leg’s dactylus (Fig. 1A) suggest a closer relationship between these two species and *P. columbiensis*, what is not in concordance with the molecular results. Nevertheless, a thick pubescence on the ventral side of the cheliped’s manus (Fig. 1B), present in *P. columbiensis* and the rest of the *P. galathinus* complex, and absent in *P. rosariensis*, *P. glasselli* and the rest of the armed-carpus species included in this clade, suggests that this character may be phylogenetically informative. The ML tree showed a relatively high PP support (82%) for the morphologically and ecologically diverse clade containing the rest of the armed-carpus species. Within this clade several clusters were well supported in both trees. One such cluster consisted of the morphologically closely related *Petrolisthes zacaе* and *P. robsonae* from the eastern Pacific, and by the amphi-American and amphi-Atlantic *Petrolisthes armatus* (specimens from the eastern Atlantic not included in this survey). This clade is consistent with morphological, physiological and ecological features shared by these three species, which occur in estuarine waters and mangrove-root systems. Two further well-supported groups corresponded to the *P. edwardsii*–*P. agassizii* group and the *P. haigae*–*P. hirtispinosus* group, both of which are morphologically consistent. A third clade containing the amphi-Atlantic *Petrolisthes marginatus* is morphologically compatible because the *P. marginatus* individuals from the western coast of Africa and the eastern American coast show no discrete phenotypic differences (see Section 4). The sequences from Cape Verde and the Colombian Caribbean differed in five transitional mutations.

3.1.3. Assessment of rates

Analysis of the entire data set rejected the molecular clock ($P < 0.005$). Additional evaluations of rate constancy were conducted for two restricted sets of taxa, one comprising all *Petrolisthes* species included in this survey, and one consisting of the complex itself (excluding *P. rosariensis*). This was conducted to investigate whether or not deviation of rate constancy might be caused by the inclusion of distantly related species. Results from the analyses restricted to *Petrolisthes* and the complex were also found to be inconsistent with the molecular clock. These results differ from those found by Stillman and Reeb (2001), who found rate constancy for three geminate pairs, *P. armatus*, *P. galathinus*, and *P. tridentatus*, using the relative rate test (Takezaki et al., 1995). The difference between the results obtained by these authors and our results may reflect differences in statistical power of the two approaches for estimating rate constancy.

We evaluated this possibility by subjecting their data to a likelihood ratio test and found the data not to meet rate constancy. The failure of this data set to meet requirements of a molecular clock, as judged by a likelihood ratio test, brings into question the use of a calibration based on *P. armatus* to evaluate times of divergence within *Petrolisthes*.

3.1.4. Genetic distances

The level of saturation in the 16S rRNA fragment was analyzed using a saturation plot (Fig. 4), in which the uncorrected *p* distances (or pairwise distances) and the distances computed using the parameters obtained from the maximum likelihood analysis, or ML distances, were contrasted. The results show a relatively good correspondence between distances, particularly for the taxa within the complex. The corrected ML distances for selected groups of taxa are presented in Table 4. For taxa represented by more

than one sequence, distance averages and corresponding ranges were calculated.

3.2. Larval morphology

For all species, for which ovigerous females were sampled, the first zoea was obtained. Only this larval stage was considered for comparison in this survey, since further development stages were achieved only for some of the species. The first zoeae of all species show the typical characters of Lebour's (1943) *Petrolisthes*-group. All larvae have typically a long rostral spine and two posterior carapace spines. Comparison of eight selected characters lead to three main larval groups of species designated as A, B, and C (Table 5). Groups A and B included larvae of species with an armed-carpus adult morphology, while group C contained the only two unarmed-carpus species included for larval comparison. Group A included the species of the *P. galathinus* complex, except *P. rosariensis*. The larvae of this group are characterized by a relatively long rostral spine (character 1), the absence of dorsal spinules on the posterior carapace spines (character 2), the lack of serration on the carapace's ventral margin (character 3), the presence of one plumose seta on the coxa of the first maxilliped (character 4), the absence of a hook on the outer margin of the first maxilliped (character 5), the setal formula 3+5 on the third segment of the endopodite of the first maxilliped (character 6), a lateral spine on the fourth abdominal somite (character 7), and bifid telsonal lateral spines (character 8). Of remarkable interest is the first zoea of *Parapetrolisthes tortugensis*, which shares all characters, except the eighth one, with all species of the *P. galathinus* complex (excluding *P. rosariensis*). Larval group B included *Petrolisthes edwardsii*, *P. haigae* and *P. marginatus*. The larvae of this group have a conspicuously long rostral spine

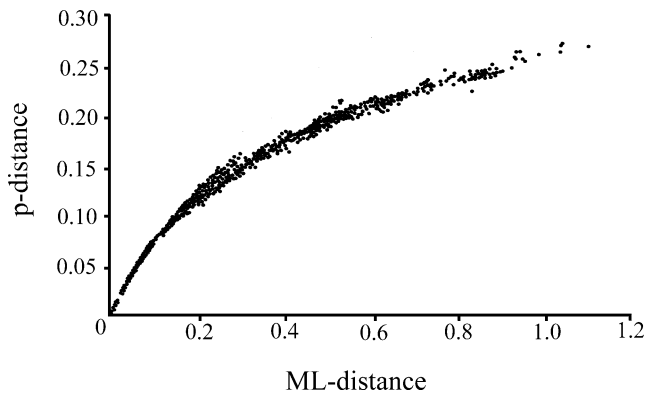


Fig. 4. Saturation plot for the complete porcellanid 16S rRNA data set. The ML distances (*X* axis), inferred using the parameters obtained from the ML analysis, are contrasted with the *p* distances (*Y* axis). See text.

Table 4

ML distances calculated for selected groups of taxa using the TV + I + G model (distances averaged when more than two sequences were compared; see text)

Group	Averaged ML distance	ML distance range
Intraspecific (nominal trans-isthmian sp. excluded)	0.003	0.002–0.009
Among different <i>P. galathinus</i> color morphs-WA	0.038	0.026–0.044
Among both <i>P. caribensis</i> color morphs	0.074	0.071–0.077
Between <i>P. armatus</i> from both American coasts	0.015	
Between <i>P. galathinus</i> forms from both American coasts	0.038	0.030–0.044
Between <i>P. tonsorius</i> from both American coasts	0.051	
Intraspecific in the amphi-Atlantic <i>P. marginatus</i>	0.090	
Among sp. in the <i>P. galathinus</i> comp. (<i>P. rosariensis</i> excluded)	0.060	0.031–0.101
Between <i>P. rosariensis</i> and the rest of the sp. in the <i>P. galathinus</i> comp.	0.243	0.201–0.274
Between <i>P. haigae</i> and <i>P. hirtispinosus</i> (two morphologically similar EP sp.)	0.063	
Between <i>P. edwardsii</i> and <i>P. agassizii</i> (two morphologically similar EP species)	0.077	
Between <i>P. armatus</i> and <i>P. robsonae</i> (two morphologically similar EP species)	0.100	0.091–0.109
Between <i>P. haigae</i> and <i>P. marginatus</i> (a nominal trans-isthmian species pair)	0.163	0.157–0.169
Between <i>P. amoenus</i> and <i>P. monodi</i> (a nominal amphi-Atlantic species pair)	0.104	
Among morph. distantly related <i>Petrolisthes</i> sp. within armed-carpus assemblage	0.252	0.159–0.338
Between <i>Parapetrolisthes tortugensis</i> and the <i>P. galathinus</i> comp. (<i>P. rosariensis</i> excl.)	0.078	0.051–0.097
Between <i>Parapetrolisthes tortugensis</i> and <i>Petrolisthes sanfelipensis</i>	0.131	
Among <i>Petrolisthes</i> sp. within unarmed-carpus assemblage	0.187	0.122–0.263
Between morphological assemblages (armed-carpus vs. un-armed carpus sp.)	0.471	0.341–0.704
Intergeneric (<i>Petrolisthes</i> and <i>Petrocheles</i>)	0.807	0.708–0.889

comp. = complex; excl. = excluded; WA = western Atlantic; EP = eastern Pacific.

correctly by comparing their individual distances with the distances of the 10 main centroids. For a clearer graphical representation of the deformations of the main consensus configuration the eight species were separated into three clusters using the k-means cluster analysis. The first cluster included *P. bolivarensis*, *P. caribensis*, *P. columbiensis*, and *P. sanmartini* and is distinguished by low standard deviations and variances. The second cluster contained the *P. galathinus* “Stripes” and eastern Pacific color forms, the *P. caribensis* “Blue” variant, *P. glasselli* and *P. rosariensis*. The standard deviations and variances are significantly higher than those of the first cluster. Considering this, a lower intraspecific variability of the sternal plate shape in the species included in the first cluster than in those of the second cluster is assumed. *Parapetrolisthes tortugensis* was located in the third cluster. The sternal plate shape of this species is considerably different from those of the *Petrolisthes* species (Fig. 5). Through the first two relative warps the four species from the first cluster can be separated with significant accuracy. The cranial part of the sternal plate in *P. caribensis* and, to a lesser degree, of *P. sanmartini*, is much narrower than that in *P. bolivarensis* and *P. columbiensis*. These species can be distinguished through a different length–width ratio of the sternal plate. *P. columbiensis*

appears to be compressed along its vertical axis, while *P. bolivarensis* is stretched along its vertical axis. *P. sanmartini* resembles the consensus configuration. A significant differentiation of the species and color forms in the second cluster was achieved through the first three relative warps. The first relative warp breaks up the cluster into four parts leaving *P. galathinus* “Stripes” and *P. caribensis* “Blue” together. *P. glasselli* showed a marked horizontal compression of the caudal region, while *P. rosariensis* displayed this deformation in a lesser degree. The *P. galathinus* color form from the Eastern Pacific showed a distinct horizontal broadening. Only the second relative warp separated *P. galathinus* “Stripes” and *P. caribensis* “Blue” because of the vertical compression of the sternal plate in *P. galathinus* “Stripes.” In summary, *P. galathinus* “Stripes” and *P. caribensis* “Blue” comprise the two most similar groups within the second cluster, while *P. glasselli* and *P. rosariensis* are clearly excluded, not only from these two variants but also from each other, with *P. rosariensis* having the most independent sternal plate shape. A hierarchic tree (Fig. 6) based on shape similarities that arrange species according to the results of the discriminant and relative warp analyses, was obtained using the method of cluster analysis. *P. bolivarensis* and *P. columbiensis* are the most

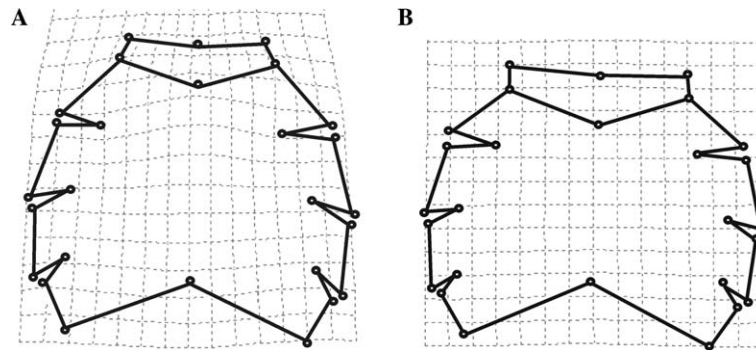


Fig. 5. Main consensus configuration of the sternal plate in: (A) *Parapetrolisthes tortugensis*, and (B) *Petrolisthes galathinus* “Stripes.” The cranial part of the sternal plate in *Parapetrolisthes tortugensis* shows a distinctive horizontal compression, which separates this species from the *Petrolisthes* species included in the geometric morphometric analyses.

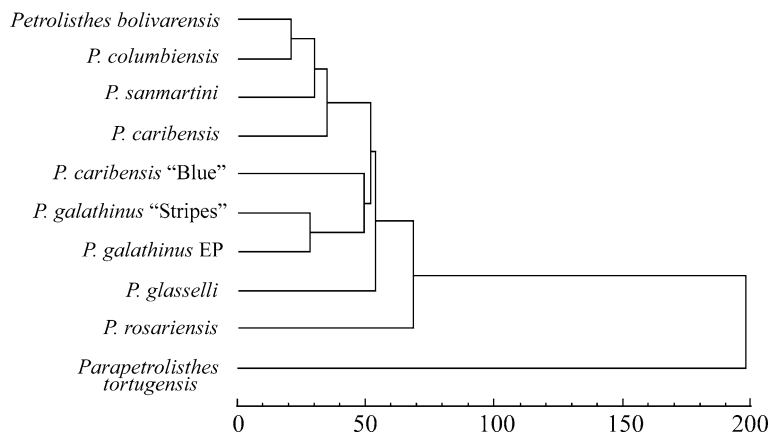


Fig. 6. Hierarchic tree based on an agglomerative cluster analysis of the Mahalanobis distance matrix shown in Table 6. As fusion rule, single linkage was used. Distance measurement was performed according to the dissimilarities in the matrix. EP = eastern Pacific.

similar species and clustered with *P. sanmartini* and *P. caribensis* in a group. The *P. galathinus* “Stripes” and the eastern Pacific *P. galathinus* color form appear to be also relatively similar and form another discrete group with the *P. caribensis* “Blue” variant. *P. glasselli* and *P. rosariensis* are positioned at the base of the *Petrolisthes* cluster, and are clearly separated from *Parapetrolisthes tortugensis*, which is located at the base of the tree.

4. Discussion

4.1. Molecules and larvae within *Petrolisthes*

Differences in adult morphology among the *Petrolisthes* species included in this survey are supported by molecular divergence among partial sequences of the mitochondrial 16S rRNA gene and by larval morphology. Nevertheless, the resulting molecular trees support the inclusion of *Parapetrolisthes tortugensis* and the exclusion of *P. rosariensis* from the clade containing the species of the *P. galathinus* complex. These results question the morphological interpretations that, on one hand, led Haig (1962) to exclude *P. tortugensis* from *Petrolisthes*, and on the other hand, led Werding (1983b) to treat *P. rosariensis* as a member of the *P. galathinus* complex. Both cases are discussed in detail below. The concordance between these molecular results and evidence from larval morphology, added to the general agreement between morphological groups within *Petrolisthes*, led us to conclude that the 16S rRNA mitochondrial gene is a relatively useful marker for inferring phylogenetic relationships among porcellanid species. Nevertheless, its use in phylogenetic studies may be limited by the heterogeneous rate at which this gene seems to evolve across the porcellanid lineage, thus hindering the calibration of a molecular clock, and a confident sequence alignment of relatively distantly related species, as observed in the preliminary analysis mentioned above.

4.2. The *P. galathinus* complex: phylogenetic relationships, taxonomic implications and the possible meaning of size and color

The molecular and geometric morphometric results presented here strongly support the validity of the six nominal species comprising the *P. galathinus* complex (Werding, 1982, 1983b; Werding and Hiller, 2002; Werding and Kraus, 2002), and of color and color pattern of live specimens to distinguish these species. Results from the molecular analysis and from larval-morphology comparisons suggest that the *P. galathinus* complex comprises five closely related or sibling species, *P. galathinus*, *P. caribensis*, *P. columbiensis*, *P. sanmartini*, and *P. bolivarensis*, and a more distantly related one, *P. rosariensis*. The specific status of each species in the complex, except *P. galathinus*, is supported by: (1) the placement of their respective DNA sequences in monophyletic groups diagnosable by coloration regardless of the geographical origin of the specimens,

(2) a unique set of haplotypes, and (3) a distinguishing shape of the sternal plate. The identity of *P. galathinus* still remains ambiguous due to the weak support of the clade containing all specimens matching this species in the MP tree, and by the inclusion of the “Blue” variant of *P. caribensis* in this clade in both MP and ML trees. A relative level of resolution detected within this clade, indicated by the monophyletic grouping of the *P. caribensis* “Blue” color form and the *P. galathinus* “White Teeth” variant, suggests the presence of two independent lineages that may represent undescribed species. Ongoing work based on sequence variation of the mitochondrial COI gene appears to support this view. So far, we can conclude that the species included in the *P. galathinus* complex can be identified by color and color pattern, contrasting the conclusions given by Stillman and Reeb (2001). In the molecular analysis of several *Petrolisthes* species these authors included 7 specimens referred to as *P. galathinus* variants 1–7 (1 collected on the Panamanian Pacific coast, and 2–7 on the Panamanian Caribbean coast), claiming to have identified two of them as *P. caribensis* and further two as *P. columbiensis* following the color descriptions given by Werding (1983b). According to the phylogenetic tree presented by these authors, within the clade exclusively containing the seven *P. galathinus* variants, only the sequences corresponding to *P. caribensis* clustered together, leading them to conclude that “coloration patterns of live specimens may not be a definitive character for species identification in all cases.” A ML analysis conducted for the complex alone, in which our sequences and the 7 sequences submitted by these authors to GenBank were included (results not shown; Accession Nos. AF260634, AF260635, AF260636, AF260637, AF260638, AF260639, and AF260623), revealed that the two variants identified as *P. columbiensis* nested together with our *P. galathinus* “White Teeth” sequences, indicating that these specimens were misidentified. *P. columbiensis* is not only characterized by a unique and uniform color form, but can be morphologically distinguished from *P. galathinus* by the presence of two instead of one epibranchial spine, and four instead of three spines on the inner side of the dactylus of the walking legs (Table 1). It is morphologically distinguishable from *P. rosariensis* (also with two epibranchial spines and four spines on the dactylus), by the presence of a conspicuous pubescence on the gap of the cheliped’s fingers (Fig. 1). These color and morphological properties were clearly highlighted by Werding (1983b) when he described *P. columbiensis*. The variants identified by Stillman and Reeb as *P. caribensis* nested in our ML tree within the clade corresponding to the *P. caribensis* “Blue” variant, which is precisely called a variant because of a color and color pattern completely different from *P. caribensis*. Variant 7 clustered with our *P. caribensis* sequences, variant 4 within our *P. galathinus* “Stripes” and “Spots” clade, and finally, the Pacific coast variant (Nr. 1), grouped as expected with our *P. galathinus* sequences from Ecuador and the Colombian Pacific coast.

So far, we can conclude that the combination of different sources of data resulted in an efficient approach for disentangling this interesting complex of species. Nevertheless, *P. galathinus* remains a complex of morphologically similar but genetically divergent lineages diagnosable through coloration and sternal plate morphology, which demands further taxonomic work and characterization through additional molecular markers.

In general, the 16S rRNA gene appears to be useful for defining species boundaries within the *P. galathinus* complex, though interrelationships among species were poorly resolved. No strong evidence for saturation, as reflected by the relatively good correspondence between corrected and uncorrected distances, implies that distortion through saturation is unlikely to affect inferences, at least among closely related species. According to this, the polytomies within the species-complex clade could be treated as hard, i.e., the species evolved within a relatively short time span. Nevertheless, failure to accept the hypothesis of rate constancy within the complex, added to an inadequate fossil record, prevent suggesting a possible radiation episode through past geological or climatic events. The only fossil similar to *P. galathinus* is *P. aviatus* described by Rathbun (1919) from the Pliocene of Central America. However, the origins of species related to *P. galathinus*, characterized by transverse pilliferous striations, date back to the Thetys fauna, as evidenced by a number of extant species from the Indo-West Pacific, e.g. *Petrolisthes decacanthus*, *P. eldredgei* and *P. moluccensis*.

An almost lack of discrete morphological differentiation among the species in the complex, contrasted to a relatively high genetic divergence, suggests that genetic and morphological divergence may be decoupled. However, the clear distinction of each species by means of the morphometric analysis of shape variation of the sternal plate suggests that genetic divergence seems to be coupled with continuous morphological differentiation. In the same way, the segregation of DNA sequences into clades diagnosable by coloration suggests that genetic divergence is coupled with different colors and color patterns in this particular porcellanid group. The apparent association between color differentiation and genetic divergence in all species within the *P. galathinus* complex suggests a link between speciation and color change. In a number of decapod crustacean groups, color and color pattern differences have proved to be useful in the identification of cryptic species (Knowlton, 1986; Knowlton and Mills, 1992), whereas their biological function (if any) remain poorly understood. In the animal realm coloration and other visual signs have played a prominent role in the theory of sexual selection. Nevertheless, in many conspicuously colored invertebrates there is no apparent sexual dimorphism, and intraspecific signaling seems unlikely to explain their coloration (Andersson, 1994). No patterns suggesting sexual selection are observed in any *Petrolisthes* species and the evolutionary significance of color and color pattern in the speciation process in porcellanid species with conspicuous colorations remains a

matter of speculation. A tendency of all species of the *P. galathinus* complex to live in hierarchically structured monospecific aggregations has been observed (own obs.). The same tendency was reported by Jensen and Armstrong (1991) for two *Petrolisthes* species from the Pacific coast of North America. Considering that the species in question have relatively large eyes, it could be assumed that vision is involved in orientation and social behavior. Meyer-Rochow and Meha (1994) reported vision to be involved in shelter seeking in *Petrolisthes elongatus*, one of the most common intertidal decapods on New Zealand shores. Within this context, and considering that the species in the complex live in sympatry, the specific coloration may play a role in conspecific recognition, thus favoring the formation and persistence of such monospecific assemblages that enhances the finding of sexual partners. On the other hand, coloration may be interpreted as an unselected by-product of adaptation to different ecological niches. In terms of competition for resources the specialized filter-feeding habit of porcellanids allows assuming that the main resource limitation is not food but available substrate, which must meet the conditions of being hard, optimally exposed regarding water movement, and offering the crabs protection from predators. From this perspective, littoral hard substrates can be basically characterized by the size of inhabitable spaces (e.g. boulders, coral interstices), by water movement in terms of exposition to currents, and by depth. Considering this, body size seems to be an important taxonomic character, though it seems to be generally underestimated in morphological comparisons. Species predominantly inhabiting coral interstices, e.g. *Petrolisthes caribensis*, *P. columbiensis*, and *P. sanmartini*, represent forms of moderate size (between 6 and 9 mm carapace length), whereas *P. bolivarensis* and the “Stripes” and eastern Pacific *P. galathinus* variants are principally boulder-dwelling species reaching carapace sizes of 14 mm and more. These clear differences in sizes in sympatric species suggest that the speciation process was probably driven by adaptation to different sizes of inhabitable space.

4.3. *Amphi-Atlantic and trans-isthmian species*

The extant tropical American porcellanid fauna arose from the tertiary Caribbean Province, which included the eastern Pacific until the closure of the Central American land bridge at the end of Pliocene (see Woodring, 1974). With that event both faunas evolved independently in allopatry. This common origin can be traced through the presence of several extant trans-isthmian species and species pairs. There is a low affinity between the American and the Indo-West Pacific faunas, while the eastern Atlantic one shows certain level of relationship with both main regions. The affinity of this fauna to the American one is evidenced by two amphi-Atlantic species (one of them also trans-isthmian) and two species pairs (see below). The amphi-Atlantic *Petrolisthes marginatus* was formerly treated as two different species, with the eastern Atlantic populations iden-

tified as *P. cesatii* until Gore (1983) synonymized this species with *P. marginatus*, based on morphological comparisons of specimens from Ascension Island and the Caribbean. On the other hand, *P. marginatus* was treated as a trans-isthmian species (Haig, 1960) until Chace (1962) described *Petrolisthes haigae* for the eastern Pacific populations. The comparison of DNA sequences of *P. marginatus* from Cape Verde and the Colombian Caribbean supports its status as an ampho-Atlantic species, considering the relatively low number of transitional mutations between these sequences. Moreover, the genetic distance between both sequences is comparable to other intraspecific distances, and is smaller than distances found within the trans-isthmian species included in this survey, what could be evidencing current or relatively recent gene-flow events among populations on each side of the Atlantic.

While the final completion of the Central American land barrier, estimated at 3–3.5 mya (see Lessios, 1998), completely interrupted gene exchange among populations of porcellanids on either side, the effectiveness of the Atlantic as a barrier to gene flow essentially depends from the species larval cycle and the existence of potential stepping stones in the central Atlantic. Scheltema (1986) estimated that larvae of marine organisms require between 9.2 and 28.3 weeks to cross the contemporary tropical Atlantic. The maximum duration of larval stages of tropical porcellanid species has been estimated between 2 and 3 weeks (see discussion in Werding and Müller, 1990), and therefore, it seems unlikely that larvae can successfully cross the Atlantic in the current oceanographic setting. Assuming that dispersion is confined only to larvae (see Werding et al., 2003), the role that oceanic islands may play in favoring dispersion seems relevant. Chace and Manning (1972) argued that Ascension is the last of a series of oceanic islands which raised and disappeared in the middle Atlantic between tropical Africa and northern Brazil. This scenario could explain a relatively recent gene exchange between western and eastern Atlantic populations of *P. marginatus*, though a more complete sampling of individuals from both sides of the Atlantic and from Ascension Island is required to further support this explanation.

Our molecular results do not support a sister-taxon relationship between *P. marginatus* and *P. haigae*, as would be expected from a morphological perspective. In the molecular trees, significantly high support was obtained for the clade containing *P. haigae*, known from the Gulf of California to Ecuador, and *P. hirtispinosus*, an endemic from the Gulf of California. Haig (1960) mentioned that *P. haigae* (then still known as *P. marginatus*) “does not appear to be well established in the Gulf [of California], where it is largely replaced by *P. hirtispinosus*.” According to Briggs (1974) a distinct warm-temperate fauna evolved from a tropical one in the Gulf of California through the development of a temperature barrier at the beginning of Pleistocene, which produced sufficient isolation to permit this process to take place. The current distributional pattern and sister-species relationship of *P. haigae* and *P. hirtispi-*

nosus, the almost undistinguishable morphology of the former species and *P. marginatus*, and a close relationship among the three species, suggest that an ancestral eurytherm population, distributed in the tertiary Caribbean province, was divided by the formation of the Central American land barrier and by the temperature barrier at the mouth of the Gulf of California. The most peripheral population, probably distributed in the Gulf, apparently split off and evolved into the warm-temperate *P. hirtispinosus*. The chronology of these events remains uncertain.

The eastern Atlantic porcellanid fauna has the lowest number of species compared to other tropical regions. Briggs (1974) suggested that contemporary temperature conditions and perhaps severe Pleistocene climatic changes are probably responsible for the relative low diversity and current distributional pattern of eastern Atlantic marine taxa in relation to other main regions. The porcellanid fauna of this region shows an Indo-West Pacific connection, as evidenced by three genera only found otherwise in the Indo-West Pacific. On the other hand, the presence of two ampho-Atlantic species and two species pairs appears to support a later immigration of western Atlantic species to the East Atlantic. Although larval migration may have occurred in both directions, an eastwards migration is assumed to have been more successful considering that the eastern Atlantic porcellanid fauna is the least diverse compared to the other main regions, probably because of adverse past climatic conditions. Furthermore, the migration of a species from a region with a high number of species to one with a lower number is expected to be more successful than a migration in the opposite direction, as immigrants would have to compete with a highly complex community of different populations perfectly adapted to local conditions (see Briggs, 1974).

One ampho-Atlantic species pair is comprised by the eastern Atlantic *P. monodi* and the western Atlantic *P. amoenus*. In all phylogenetic reconstructions presented here, these species show a well supported sister-species relationship that is consistent from a morphological perspective because the two species are virtually identical.

The American porcellanid fauna counts with six nominal trans-isthmian species and several species pairs. The trans-isthmian or species-pair status in porcellanids has mainly depended on the identification of constant discrete morphological characters in adults or larvae that give argumentation for species distinctiveness. Haig (1960) recommended for virtually undistinguishable species pairs to be studied in more detail to determine whether the eastern and western American populations should both be accorded full specific rank. Nevertheless, populations not showing discrete morphological differences may be clearly distinguished through other criteria like coloration, shape variation of different body structures, or molecular divergence, which may be reflecting an advanced stage of genetic isolation. *P. galathinus* from the eastern Pacific was described by Stimpson (1858) as *P. occidentalis* but was later synonymised with the western Atlantic populations (reviewed by

Haig, 1960). The two transversions supporting the clade containing the eastern Pacific individuals from Colombia and Ecuador, added to a unique coloration and geometric morphometric distinction, advocate the specific rank of the eastern Pacific populations. Therefore, we recommend Stimpson's *P. occidentalis* to be re-established. The second trans-isthmian species included in this survey, *Petrolisthes tonsorius*, was first described from the eastern Pacific (Haig, 1960) and found later in the western Atlantic (Werding, 1977). *P. tonsorius*, as well as *P. quadratus* and *P. tridentatus* belong to a group of species with very few diagnostic characters, as they lack any spination or presence of teeth on the carapace, chelipeds or walking legs. Specimens of *P. tonsorius* from both sides of the Central American land bridge are morphologically undistinguishable and show no geographically localized variation in color or color pattern. The genetic distance (0.051) between the *P. tonsorius* individuals from both American coasts is in general smaller than the distances between morphologically close but well distinguishable species (e.g. *P. edwardsii*–*P. agassizii*; see Table 4). Although this fact could support the treatment of *P. tonsorius* from both coasts as one species, genetic distances should not be used as a unique criterion to diagnose species (see below). Further evidence from a geometric morphometric analysis and from a survey of divergence among DNA-sequences of other genes (e.g. the mitochondrial COI gene) are to be accomplished to determine if the western Atlantic *P. tonsorius* should be recognized as a new species.

P. armatus is the only porcellanid species with a neotropical and subtropical distribution and a geographic range including both sides of the Atlantic and the eastern Pacific. Specimens from both sides of the Central American land bridge show the smallest genetic distance (0.015) in relation to those found in the other two trans-isthmian species discussed above. The extraordinary ecological plasticity of this species, reflected in its occurrence in a wide range of habitats including estuarine waters, as well as a wide depth range and tolerance to different climatic conditions, could explain its wide geographic range (see Werding et al., 2003), and may have enabled populations on both sides of the Central American land bridge to be connected for a longer time than species ecologically more restricted. On the other hand, populations from different localities show considerable morphological variation indicating that *P. armatus* may prove to be a species complex.

The comparison of genetic distances among and within species discussed above was intended to have an overview of the correlation between morphological and molecular divergence in well established and in problematic porcellanid species, rather than to try establishing a reference threshold for the 16S rRNA gene for determining species boundaries. It is important to notice that genetic distances should not be used as a unique criterion to diagnose species, as it is not parsimonious and its theoretical foundations are poorly understood (Ferguson, 2002). Furthermore, the lack of rate constancy observed in our

16S rRNA data set makes the comparison of genetic distances among different lineages even more questionable. Therefore, it would be inadequate to conclude that Pacific and Atlantic populations of a nominal trans-isthmian species, not distinguishable through morphological differences (e.g. *Petrolisthes tonsorius*), but showing similar genetic distances as those observed for a well established species pair (e.g. *P. haigae* and *P. hirtispinosus*), should be considered as different species based on this criterion only.

4.4. *Parapetrolisthes tortugensis* and *Petrolisthes rosariensis*: unexpected results from a morphological perspective and their taxonomic implications

From an adult-morphological perspective the lack of molecular support for a close relationship between *Petrolisthes rosariensis* and the rest of the species in the complex, compared to a relatively well supported close relationship between the complex and *Parapetrolisthes tortugensis*, appear most unexpected. Interestingly, the larval morphology of the two species suggests that this unforeseen placement may be correct. The resemblance of the adult morphology of *P. tortugensis* with species of the galatheid genera *Galathea* and *Munida* lies principally in the combination of the typical galatheid-like long pincer-shaped fingers of the chelipeds, a relatively less depressed body form, as compared with other *Petrolisthes* species, and the conspicuous spination of carapace and chelipeds. The distinctive body form of *P. tortugensis* is clearly manifested in a significantly different sternal plate shape, compared to *Petrolisthes* species (Fig. 5). Contrasting the molecular and larval results, which suggest a close evolutionary relationship between *P. tortugensis* and the *P. galathinus* complex, the particular form of the sternal plate in this species reflects one of several morphological adaptations to new ecological demands (see below). Unlike porcellanids, which filter-feed through the use of their highly adapted third maxillipeds, galatheids have been reported to use many different food resources using their pincer-shaped fingers of the chelipeds to select, tear and transport food to the maxillipeds (Garm and Høeg, 2000; Hudson and Wigham, 2003), and their setose third maxillipeds to sweep detritus from the substratum, passing material on to other mouth parts (Hall-Spencer et al., 1999). Milne-Edwards and Bouvier (1894; reviewed in McLaughlin and Lemaitre, 1997) interpreted the morphological variations seen in most galatheoidea as adaptations to habitat and depth. The general morphology of *Parapetrolisthes tortugensis* may reflect ecological adaptations to a different habitat compared to that occupied by the flattened-body *Petrolisthes* species, which can be interpreted as a hydrodynamic adaptation to strong water movement in the upper littoral. The almost complete lack of functionality of the chelipeds for feeding purposes in *Petrolisthes* supports this tendency. Contrastingly, *P. tortugensis* occurs associated to coral rubble at greater depths (down to 40 m) and under low water-movement conditions, where other porcellanids rarely occur. This situation results

in suboptimal conditions for filtering, what is complemented by the use of the chelipeds for feeding (Werdinger, pers. obs.). The less flattened carapace form, characteristic of *P. tortugensis*, supports this view. In the light of evidence provided by the molecular and larval data it is possible to conclude that the galatheid-like habitus of *Parapetrolisthes* does not constitute a reminiscent morph of a number of forms among the Galatheidae, as suggested by Glassell (1945), but rather reflects morphological convergences among non-closely related species, resulting from adaptation to similar habitat conditions. As substrate availability for optimal filtration seems to be the most competed resource (see above) it is possible that *Parapetrolisthes* conquered a less attractive but less competed, and possibly unoccupied habitat that demanded considerable morphological adaptations for its optimal exploitation. The view that *P. tortugensis* is a highly derived animal is supported by the molecular character analysis showing a large number of autapomorphic traits (16).

Regarding the taxonomic status of this species we consider it not to merit generic distinction. This will be taken into consideration once the revision of *Petrolisthes* is accomplished on a world-wide basis.

Concordant differences showed by independent sources of data appear to indicate that *P. rosariensis* does not share a recent common ancestor with the rest of the species in the complex. Particularly, the morphology of the first larval stage of this species, showing more characters in common with larvae of other species within the *P. galathinus*–*P. lamarckii* group than with those of the rest of the species in the complex, seems to support this view. The molecular phylogenies indicate a sister-taxon relationship between *P. rosariensis* and *P. glasselli*. This eastern Pacific species has a very similar morphology, color and color pattern to that of *P. columbiensis*. However, *P. glasselli* can be distinguished by the absence of pubescence in the gape of the fingers of the chelipeds, and by an additional tooth on the cheliped's carpus.

The high level of 16S rRNA sequence divergence shown between the *P. rosariensis*–*P. glasselli* group and the rest of the species in the *P. galathinus* complex is consistent with morphological stasis. The characteristic morphology of *P. galathinus* and related forms is observed in Indo-West Pacific species, suggesting that extant American and Indo-West Pacific species with this general morphology probably arose in a Thetyan ancestor (see above). The Indo-West Pacific forms have been reported to occur in similar habitats as the American species (see Haig and Kropp, 1987), i.e., complex systems of interstices formed by corals and boulders moderately exposed in the upper littoral. This could imply that the retention of this plesiomorphic condition resulted in an optimal and efficient exploitation of such a habitat. This suggestion is highly speculative, and further morphological comparisons and molecular approaches including Indo-West Pacific species are necessary for understanding the ecological circumstances under which this apparent morphological stasis appears to be favored.

4.5. The position of *Petrocheles* within *Porcellanidae*

The general adult morphology of *Petrocheles* gives the impression of a galatheid-like porcellanid, as noted by Wear (1965). Referring to *P. australiensis* Baker (1905) mentioned that “It is impossible to look at this species without recognizing its strong likeness to the family Galatheidae.” Hale (1927) also mentioned the strong resemblance of *Petrocheles* to some members of this family. This similitude is based mainly on the carapace shape, carapace and chelipeds spination, a relatively developed front, and a less folded abdomen, giving the impression of a less advanced carcinization state as in the rest of porcellanids. The life cycle and larval morphology of *Petrocheles* suggests a close relationship between this genus and galatheids, because it undergoes five, instead of the typical two zoeal stages known for porcellanids, and because some aspects of the zoeal morphology are shared by the two taxa (Wear, 1965; see Gurney, 1924). On the other hand, *Petrocheles* presents the typical porcellanid filter-feeding third maxillipeds lacking an epipodite (present in galatheids). Nevertheless, some characters make *Petrocheles australiensis* different from its congener and from the rest of porcellanids, as are the shape and plate composition of the telson and the presence of well developed pleopods on the first and second abdominal segments in males (porcellanids usually have one pair on the second segment).

Our molecular results provide independent support for the particular systematic position of *Petrocheles*. All tree reconstruction methods showed support for the exclusion of *Petrocheles australiensis* from the porcellanid clade, though the sister-species relationship between this species and *G. squamifera* probably results from incomplete galatheid taxon sampling. Further studies including *Petrocheles spinosus* and additional porcellanid and galatheid species should give more light into the phylogenetic relationships between this genus and both galatheoid families.

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