Redescription of *Petrolisthes edwardsii* (de Saussure) and description of a new, sibling species from the eastern Pacific based on different colour, morphology and genetic identity (Crustacea: Anomura: Porcellanidae)

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Abstract

Petrolisthes edwardsii (de Saussure, 1853) has been viewed as a morphologically variable species with a wide distribution in the tropical and subtropical eastern Pacific. The original description of this species is rather inaccurate, and for more than a century there has been confusion regarding the final repository of type specimens. Material recently collected in the tropical eastern Pacific revealed two different colour morphs, also distinguishable through subtle discrete and continuous differences. Detailed examination of the adult morphology of these two morphotypes and phylogenetic analysis of DNA sequences of a fragment of the mitochondrial COI gene were consistent in the separation of the two forms, irrespective of the geographic origin of the specimens. Therefore, we treat these forms as different species. One form is newly described as *Petrolisthes donadio* n. sp., the other redescribed as *P. edwardsii*. The two species live in sympatry across most of their geographic range, and occupy similar habitats. A possible geographical mode of speciation is discussed.
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Introduction

Porcellanids are marine crab-like decapods, typically littoral or sublittoral, with most species distributed in tropical regions. The eastern Pacific fauna is one of the richest, with 92 of ∼260 species recognised worldwide (Haig 1960; Hiller et al. 2004). *Petrolisthes* Stimpson, 1858 is the largest and most morphologically diverse genus in the family, with nearly 100 species worldwide. Ortmann (1897) divided it into different natural groups, based on comparative adult morphology. Haig (1960) redefined those groups, establishing five morphological lines into which most *Petrolisthes* species tend to fall. *Petrolisthes edwardsii* (de Saussure, 1853) belongs to the *P. galathinus–P. lamarckii* group, which is characterised by teeth or spines on the chelipeds, carapace and walking legs. This group is interesting from an evolutionary and biogeographic perspective, as it contains sympatric and allopatric species pairs in the eastern Pacific, and across the Isthmus of Panama and the Atlantic, some of which constitute sibling species diagnosable by conspicuous colourations and subtle morphological differences (see Hiller et al. 2006). Therefore, this group of porcellanids is ideal for

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conducting evolutionary studies on the role of intrinsic and extrinsic barriers in speciation processes.

Sibling species, i.e. reproductively isolated species that are "near the invisible end of the spectrum of morphological species differences" (Mayr and Ashlock 1991), appear to be common in several groups of marine taxa (Knowlton 1993). Molecular markers have extraordinary potential for diagnosing sibling species and other closely related taxa, where morphological or other traditional markers are ambiguous or have failed (Avise 1994). The mitochondrial COI gene has proven useful in identifying previously unrecognised species and in giving taxonomic resolution within different groups of decapod crustaceans (Sarver et al. 1998; Mathews et al. 2002; Pérez-Losada et al. 2002; Macpherson and Machordom 2005).

P. edwardsii was described by de Saussure (1853) as *Porcellana edwardsii*, from a small collection of crabs from Mazatlán, Mexico. It is one of several porcellanid species with a wide distributional range in the eastern Pacific, reported from the Bay of Santa María, Baja California, and Puerto Peñasco, Gulf of California, to Salinas, Ecuador (Hiller et al. 2004).

Apparently, de Saussure did not designate any type material, and its final repository has been a matter of confusion for more than a century. Boyko (2000) presented three *P. edwardsii* specimens from Mazatlán, deposited in the Academy of Natural Sciences of Philadelphia (ANSP), as probable syntypes. One of the clues followed by this author is a list of specimens from Mazatlán, Mexico, donated by a member of the ANSP, Dr. Thomas Bellerby Wilson, before 1857. However, the list included all taxa cited by de Saussure (1853), except *Porcellana edwardsii*, which Boyko added to Wilson's list, arguing that "Because no other likely candidates for types of this taxon have been found, and because Wilson did not always donate all the taxa from the same author or publication together … it seems very probable that these are the syntypes … donated to ANSP by Wilson". While looking for type material of *P. edwardsii* we were referred to ANSP (C.B. Boyko, personal communication 2004), from where these three specimens were sent to us for examination. This material is extremely fragile and partially fragmented because of its dry-conservation.

Haig (1960) was the first to clearly allude to the intraspecific morphological variation in *P. edwardsii*. She mentioned the presence or absence of a supraocular spine, highlighting that "at least its position [is] clearly marked by a small lobe", and referred to the proximal tubercles along the outer margin of the cheliped's manus as "usually" produced into spines.

Material sampled during a recent field trip to the Pacific coast of Colombia revealed two different colour forms morphologically matching *P. edwardsii*. These forms were collected in sympatry. A more careful examination of the Colombian material led us to discover constant morphological differences corresponding to each colour morph, allowing for a clearer distinction of the two forms. One of them, designated as "Violet" because this colour predominates on the carapace and extremities, lacks a supraocular spine and has a relatively more robust appearance. The other form was designated as "Orange", because the distal segments of the walking legs are bright orange, and the background of carapace and chelipeds has a greater amount of orange than violet; this form has a supraocular spine, and compared to the "Violet" form, it has a more gracile appearance. Differences in colour pattern, most evident on the distal segments of the walking legs (carpus, propodus and dactylus), and additional morphological characters further distinguish the "Violet" from the "Orange" form. Despite these differences, distinction of the two morphotypes becomes ambiguous if colouration has faded in alcohol-preserved material. Judging from personal collection data and museum material, the geographic distributions of the two forms overlap broadly and their ecological requirements are similar.

Colour photographs of the two species can be viewed at: http://www.uni-giessen.de/porcellanidae/#pictures1.

In the present study we analysed in detail the adult morphology of the two *Petrolisthes* morphotypes by examining museum and personal material from different localities along the American Pacific coast. Additionally, a phylogenetic analysis of DNA sequences of the mitochondrial COI gene was conducted. The resulting phylogenetic trees were used to explore boundaries between the two *P. edwardsii* forms, to evaluate their species status, and to corroborate the validity of colour and colour pattern for their distinction. Results showed two clearly separated clades that support species status for each morphotype. Therefore, we describe one of them as a new species. Because the morph lacking a supraocular spine, which matches the "Violet" variant, corresponds to the one drawn by de Saussure (1853, pl. 12, fig. 3), and because it seems to be better documented than the other form (Boone 1932, fig. 10; Haig 1960, pl. 21), we propose to assign it as *P. edwardsii*. We provide a redescription of this species, considering the brief description and poorly detailed drawing given by its author as well as the confusion concerning the type material. Among the three probable syntypes present in ANSP (see above), one corresponds to the morphotype lacking a supraocular spine, and the other two to the form with such a spine. Therefore, we accept Boyko's (2000) argument for type status and designate the ANSP specimen of *P. edwardsii* as the lectotype.

Material and methods

Specimens were collected in different localities of the Colombian Pacific, including Gorgona and Malpelo

Islands, and in Ecuador, between 1979 and 2004. Colouration of fresh specimens was documented prior to preservation. Museum material was made available for morphological examination by the Natural History Museum of Los Angeles County (LACM), the Museum of Comparative Zoology — Harvard University (MCZ), the Zoological Museum — University of Copenhagen (ZMUC), the ANSP, and the Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt a.M. (SMF). For the synonymy of *P. edwardsii* only those references published after Haig's monograph (1960), and which represent new contributions to the taxonomy, biogeography and/or ecology of the species, were included. For the molecular analysis, part of the material collected between 2000 and 2004 was preserved in 95% alcohol and stored at −20 °C until proceeding with DNA extraction. A cheliped of a *P. edwardsii* specimen from Galapagos was donated by the crustacean collections of the LACM for molecular analysis. Specimens examined from personal and museum material are listed in Appendices A and B.

In order to test whether *P. edwardsii* and the new putative species significantly differ in the length of the propodi of the walking legs, the ratio between carapace length and propodus length of all walking legs was calculated in individuals of each species, and were compared using a Mann–Whitney U-test.

Molecular techniques and analysis

DNA from 22 individuals (listed in Table 1) representing *P. edwardsii*, the new putative species and the outgroup (see below), was extracted from the chelipeds using the Qiagen DNeasy® Kit, following the protocol for animal tissues. A region of about 640 bp of the mitochondrial COI gene was amplified using the primers CO1a-H and CO1f-L (Palumbi et al. 1991). Double-stranded amplifications were performed in 25 µl volume reactions containing 10x Taq buffer, Taq polymerase, 1.3 mM dNTPs, each primer at 20 µM, and ddH2O. PCR conditions consisted of an initial denaturation at 96 °C for 3 min, followed by 40 cycles of 95 °C for 1 min, 53 °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 sequencing facility Genterprise GmbH, Mainz, Germany. *Petrolisthes armatus* (Gibbes), a member of the same morphological group within *Petrolisthes* that contains *P. edwardsii*, was selected as outgroup. Table 1

Table 1. Specimens of three *Petrolisthes* species sequenced for a fragment of the COI gene, with respective morphotype (determined by colouration and presence/absence of a supraocular spine = SocSp), collection data, sequence designation in the molecular dataset (see also Fig. 7), and GenBank accession number

Species	Colour	SocSp	Collection data	Sequence designation	GenBank No.
P. edwardsii	Violet	−	Col, Arusí, 0.5–1 m (low tide), ix.2003	*edwardsii*-Violet-Col_coast1	DQ525010
P. edwardsii	Violet	−	Col, Arusí, 0.5–1 m (low tide), ix.2003	*edwardsii*-Violet-Col_coast2	DQ525011
P. edwardsii	Violet	−	Col, Arusí, 0.5–1 m (low tide), ix.2003	*edwardsii*-Violet-Col_coast3	DQ525012
P. edwardsii	Violet	−	Col, Arusí, 0.5–1 m (low tide), ix.2003	*edwardsii*-Violet-Col_coast4	DQ525013
P. edwardsii	Violet	−	Col, Malpelo Isld., El Arrecife, 6 m, 29.i.2002	*edwardsii*-Violet-Col_Malp1	DQ525014
P. edwardsii	Violet	−	Col, Malpelo Isld., El Arrecife, 6 m, 29.i.2002	*edwardsii*-Violet-Col_Malp2	DQ525015
P. edwardsii	Violet	−	Col, Malpelo Isld., El Arrecife, 12–15 m, ix.2004	*edwardsii*-Violet-Col_Malp3	DQ525016
P. edwardsii	Violet	−	Col, Gorgona Isld., Azufrada, 0.5 m (low tide), iii.2000	*edwardsii*-Violet-Col_Gorg1	DQ525017
P. edwardsii	Violet	−	Col, Gorgona Isld., Azufrada, 0.5–1 m (low tide), iii.2000	*edwardsii*-Violet-Col_Gorg2	DQ525018
P. edwardsii	Violet	−	Col, Gorgona Isld., Azufrada, 0.5–1 m (low tide), iii.2000	*edwardsii*-Violet-Col_Gorg3	DQ525019
P. edwardsii	Presvd.	−	Ecu, Galapagos Islds., LACM CR 1934-207.6	*edwardsii*-Pres-Ecu_Galap	DQ525020
P. donadio	Orange	+	Col, Gorgona Isld., Azufrada, 0.5–1 m (low tide), iii.2000	*donadio*-Orange-Col_Gorg1	DQ525000
P. donadio	Orange	+	Col, Gorgona Isld., Azufrada, 0.5–1 m (low tide), iii.2000	*donadio*-Orange-Col_Gorg2	DQ525001
P. donadio	Orange	+	Col, Gorgona Isld., Azufrada, 0.5–1 m (low tide), iii.2000	*donadio*-Orange-Col_Gorg3	DQ525002
P. donadio	Orange	+	Col, Arusí, 0.5–1 m (low tide), ix.2003	*donadio*-Orange-Col_coast1	DQ525003
P. donadio	Orange	+	Col, Arusí, 0.5–1 m (low tide), ix.2003	*donadio*-Orange-Col_coast2	DQ525004
P. donadio	Orange	+	Col, Arusí, 0.5–1 m (low tide), ix.2003	*donadio*-Orange-Col_coast3	DQ525005
P. donadio	Orange	+	Col, Arusí, 0.5–1 m (low tide), ix.2003	*donadio*-Orange-Col_coast4	DQ525006
P. donadio	Orange	+	Col, Malpelo Isld., El Arrecife, 12–15 m, ix.2004	*donadio*-Orange-Col_Malp	DQ525007
P. donadio	Orange	+	Ecu, Salinas, 1 m, i.2002	*donadio*-Orange-Ecu_coast1	DQ525008
P. donadio	Orange	+	Ecu, Salinas, 1 m, i.2002	*donadio*-Orange-Ecu_coast2	DQ525009
P. armatus			USA, FL, Fort Pierce, Harbor Branch, intertidal, vi.2001	*Petrolisthes armatus*	DQ525021

All material collected by the authors and collaborators (see acknowledgements), except for the *P. edwardsii* specimen from Galapagos (Ref. No. VIII63) donated by LACM and of unknown colouration (presvd. = preserved; see text). Col = Colombia; Ecu = Ecuador; FL = Florida; Galap = Galapagos; Gorg = Gorgona; Malp = Malpelo.

lists all specimens sequenced for this survey. Sequences were submitted to the GenBank database under accession numbers DQ525000–525021. Sequence edition was carried out using the BioEdit Sequence Alignment Editor (Hall 1999). Sequences were aligned using the ClustalW multiple-alignment program (Thomson et al. 1994) implemented in BioEdit. Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed with PAUP* version 4.0b8 (Swofford 2001). This program was also used to calculate uncorrected pairwise distances (*p*) for all possible pairs of sequences. The MP analysis consisted of heuristic searches with 50 random-addition replicates and the TBR branch-swapping option. The bootstrap method (Felsenstein 1985) was used to estimate nodal support in the MP results (1000 replicates). Decay indices (Bremer 1988) were calculated to assess clade reliability in the resulting MP trees, using MacClade version 4.02 (Maddison and Maddison 2001) and PAUP*. MacClade was also used to examine type and distribution of substitutions supporting clades in the trees. For the ML analysis an unconstrained model was estimated using the MP majority-rule consensus tree as input. The estimated parameter values were used to conduct a full likelihood analysis. A Bayesian analysis was performed using MrBayes3 (Ronquist and Huelsenbeck 2003), with 6 free parameters for the model and 2 extra free parameters, one for proportion of invariable sites and one for the gamma distribution. Chains were run for 2,000,000 generations and the first 200,000 were discarded as burnin. The remaining trees were used to estimate posterior probabilities to determine clade credibility. PAUP* was used to subject the dataset to a test of rate constancy through a likelihood-ratio test (Felsenstein 1988). The sister-species status of *P. edwardsii* and the new species was confirmed in a separate analysis (results not shown). This consisted of the same approaches to phylogenetic inference mentioned above applied to a set of sequences of the mitochondrial 16S rRNA gene. The dataset included 10 additional *Petrolisthes* species selected according to increased levels of divergence from *P. edwardsii* and the new putative species.
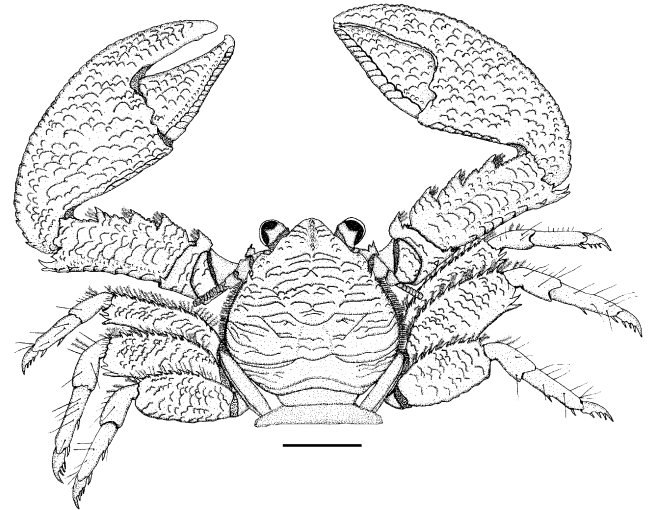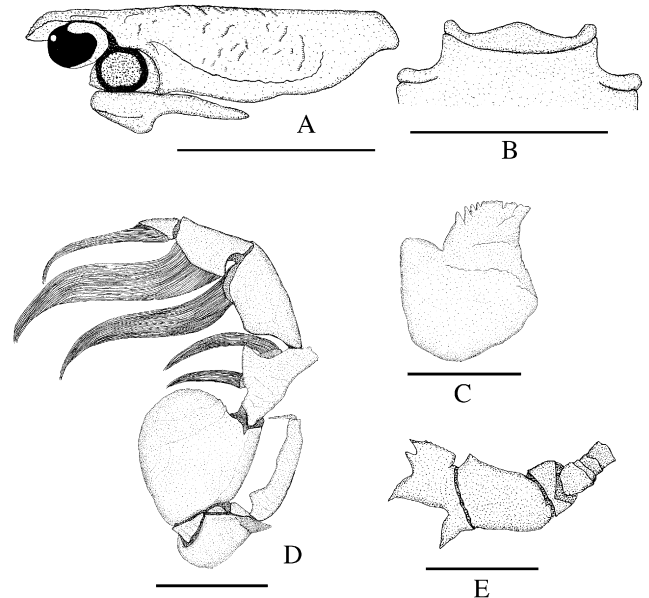
Results

Systematic account

Petrolisthes edwardsii (de Saussure, 1853)
(Figs. 1–3)

Porcellana edwardsii de Saussure, 1853: p. 366, pl. 12, fig. 3

Petrolisthes edwardsii (de Saussure): Haig (1962, p. 175; 1968, pp. 60–61); Brusca and Haig (1972,

Fig. 1. *Petrolisthes edwardsii* (de Saussure), male, INV-CRU 5777; dorsal view. Scale: 5 mm.

Fig. 2. *Petrolisthes edwardsii* (de Saussure), ovigerous female, INV-CRU 5778. (A) Carapace, lateral view. (B) Anterior thoracic sternites. (C) Basal segment of left antennula. (D) Left third maxilliped. (E) Right antenna. Scale: A and B = 3 mm; C and E = 1 mm; D = 2 mm.

p. 56); Gore and Abele (1976, p. 21); Gore (1982, pp. 12–13); Boyko (2000, pp. 126, 129–130, text-fig. 7); Hiller et al. (2004, pp. 4–5).

Material examined (including lectotype designation). See Appendix A.

Measurements (carapace length x width). Largest male 17.3 × 18.8 mm; largest female 11.2 × 11.2 mm.

Description. Carapace as long as broad, weakly convex front to back and transversely, and broadest on midbranchial to postero-branchial region. Surface with strong transverse piliferous striations interrupted at
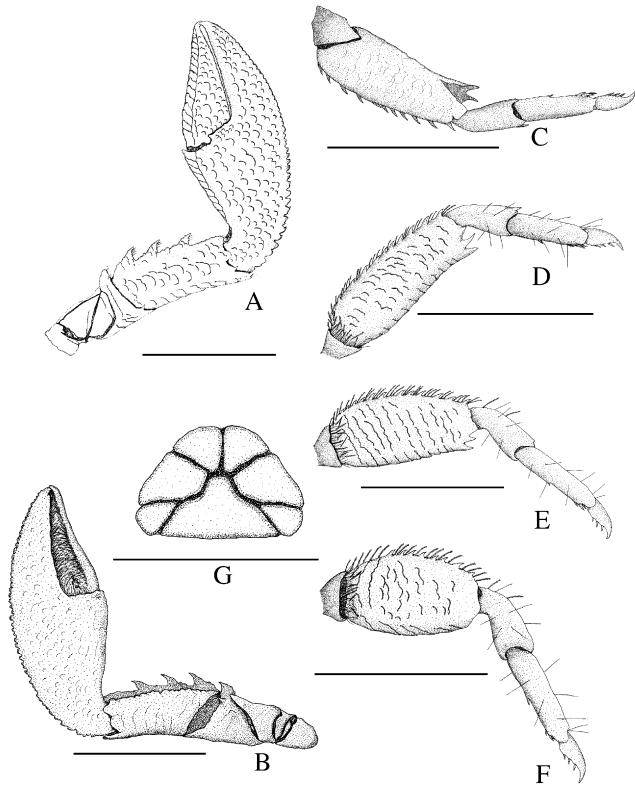
Fig. 3. *Petrolisthes edwardsii* (de Saussure), ovigerous female, INV-CRU 5778. (A) Right cheliped. (B) Right cheliped, ventral view. (C) Right first walking leg, ventral view. (D) Right first walking leg. (E) Right second walking leg. (F) Right third walking leg. (G) Telson. Scale: A–F = 5 mm; G = 3 mm.

the cervical groove, and at metabranchial regions; all regions well marked; a single strong epibranchial spine present. Front triangular with a median longitudinal depression, its borders fringed by evenly set, minute granules; orbits moderately deep, supraocular spine absent, its position marked by a smoothly elevated lobe; postorbital angle scarcely produced, not spined. Lateral borders of carapace fringed with plumose setae, lateral walls covered with such setae and with some longitudinal ridges. Third thoracic sternite convex posteriorly, trilobite anteriorly. Telson seven-plated. Eyes large. Anterior margin of basal segment of antennulae usually with 4 blunt spines, the anterodistal angle produced into a broad, spoon-like lobe. First movable segment of antennae with a strongly projecting, serrate-edged, lamellar lobe, bearing 1 or 2 spinules; second granular, and anterodistal angle projected with a conical projection; third nearly smooth, with a median tooth-like lobe on terminal margin; flagellum naked. Ischium of third maxilliped broad, rounded, laterodistal edge with a brush of feathered setae (omitted in Fig. 2D); merus triangular, with laterodistal angle usually tipped with a blunt spine; carpus with a few longitudinal plications. Chelipeds subequal, ischium with some

piliferous plications; merus with transverse piliferous striations, with serrate, spine-tipped lobe on anterior margin, flexor margin with 1 or 2 forward-directed spines towards posterior angle and an additional one on medial surface of posterior half; ventral surface with either a strong or blunt subterminal spine on anterior half; carpus more than twice as long as broad, armed on anterior margin with 3 low, broad, serrate-edged teeth, all spine-tipped; surface with large, flattened piliferous tubercles, median ones largest and forming a longitudinal row; some tubercles along posterior margin produced into spines (0–7, usually less than 3); distal angle projected into 2 superimposed prominent spines. Manus broad, flattened, covered with tubercles similar to those of carpus, but more rounded; outer margin of manus convex, with a row of tubercles, the most proximal ones produced into blunt spines (0–9, usually less than 3); inner margin with row of strongly marked tubercles; outer margin of movable finger with row of strongly marked tubercles. Ventral surface of chelipeds with tubercle-shaped plications; gape of fingers with an extended short, thick pubescence. Walking legs with small, flattened tubercles; anterior margin of merus with a fringe of plumose setae, all segments with irregularly set, long, simple setae; anterior margin of merus of leg one with 9–11 spines, of leg two with 10–12, of leg three with 5–11; posterior margin of merus of legs one and two with 1 or 2 (rarely 3) posterodistal spines; propodus with a median spine on ventral surface and a terminal triplet of movable spines on ventral border; ratio between the lengths of carapace and propodus of leg one ranging from 2.49 to 3.43 (average 2.91), of leg two from 2.09 to 2.77 (average 2.33), of leg three from 2.09 to 2.67 (average 2.51); dactylus with 3 movable spinules on inner border.

Colouration. Striations of carapace marked by dark purple, broad bands on a light lilac background; cervical groove light brown; epibranchial spines dark reddish purple; all segments of antennae with a light pink, irregular stripe which extends along segments, flanked by broad, dark brownish-purple bands along anterior region, and more irregularly along posterior one; all segments of antennae dark brownish purple along anterior and sometimes posterior regions, and with a light pink, irregular stripe along posterior or median region; serrate lamellar lobe of first movable segment light blue, with dark reddish purple margins; flagellum brownish purple; flattened tubercles of chelipeds dark reddish purple at the tip on a light pink and lilac background, the three low, broad serrate-edged teeth with white spines at the tips; outer margin of manus with dark reddish purple tubercles interrupted by short, brown, transverse stripes; ventral surface of chelipeds reddish purple with purple and some orange spots; gape pubescence orange; ischium, merus and carpus of third maxillipeds purple with dark orange background;

propodus and dactylus, as well as antennulae, blue with purple spots. Merus of walking legs light brown, with flattened tubercles reddish purple; carpus light brown proximally and with a reddish purple band distally; approximately first third of propodus light-brown banded, the remaining two-thirds with a reddish purple band and a subterminal, narrow orange band towards flexor margin; dactylus with a wide reddish-purple band flanked by narrow white stripes. Walking legs reddish purple in ventral view.

Biology. Based on personal and museum collection data *P. edwardsii* occurs from the lower intertidal to 15 m depth, predominantly under stones. Ovigerous females have been collected in every month from December through February, and in September and October.

Geographic range. Petrolisthes edwardsii ranges from the mouth of the Gulf of California to the northern coast of Peru, and is present in the Cocos, Malpelo and Galapagos Islands. Further examination of material is needed to complement the geographic range of this species.
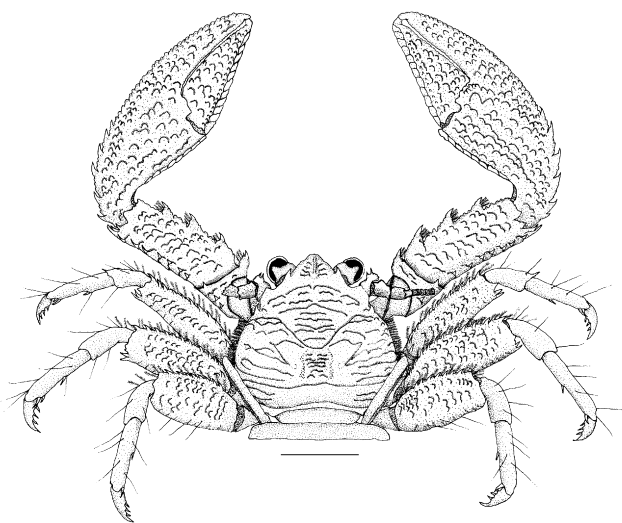
Petrolisthes donadio n. sp.
(Figs. 4–6)

Etymology. The species is named in honour of Alberto Donadio, for his unconditional help and support to AH in her academic career.

Material examined (including type designations). See Appendix B.

Measurements (carapace length x width). Largest male 15.2×16.6 mm; largest female 13.1×14.1 mm; holotype female 10.3×10.9 mm.

Description. Carapace about as long as broad, in most specimens slightly broader than long; weakly convex

Fig. 4. *Petrolisthes donadio* n. sp., holotype, ovigerous female, INV-CRU 5780; dorsal view. Scale: 5 mm.
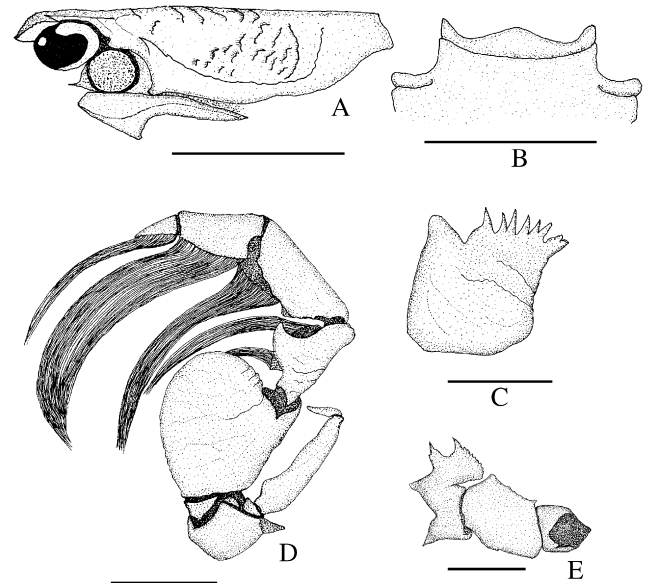
Fig. 5. *Petrolisthes donadio* n. sp., paratype, female, INV-CRU 5782. (A) Carapace, lateral view. (B) Anterior thoracic sternites. (C) Basal segment of left antennula. (D) Left third maxilliped. (E) Right antenna. Scale: A and B = 5 mm, C and E = 1 mm, D = 2.5 mm.
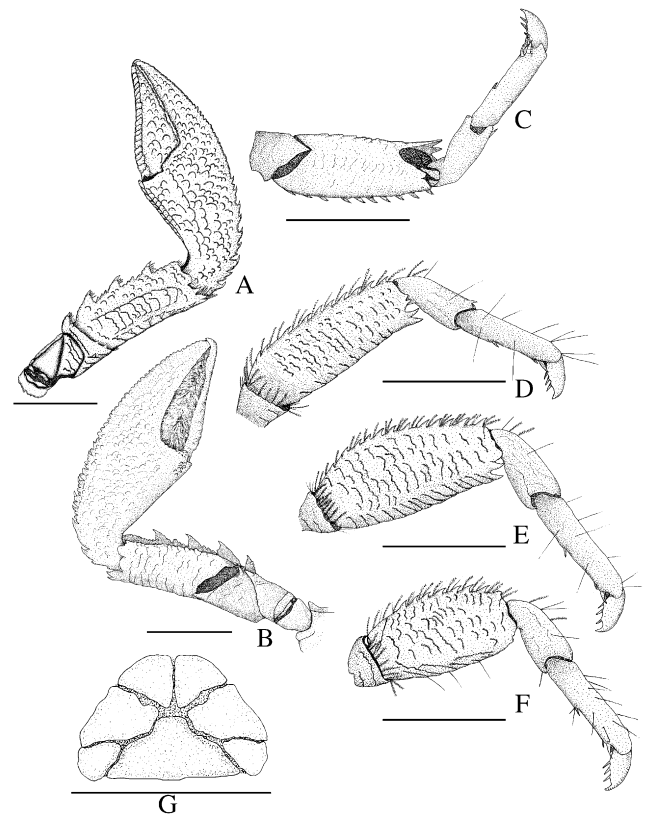
Fig. 6. *Petrolisthes donadio* n. sp., paratype, female, INV-CRU 5782. (A) Right cheliped. (B) Right cheliped, ventral. (C) Right first walking leg, ventral view. (D) Right first walking leg. (E) Right second walking leg. (F) Right third walking leg. (G) Telson. Scale: 5 mm.

front to back and transversely, and broadest at midbranchial to postero-branchial region. Surface with strong transverse piliferous striations interrupted at the cervical groove, and at metabranchial regions; all regions well marked; a single strong epibranchial spine present. Front triangular with a median longitudinal depression, its borders fringed by evenly set, minute granules; orbits moderately deep, supraocular spine present, frequently blunt in larger individuals; post-orbital angle scarcely produced, not spined. Lateral borders of carapace fringed with plumose setae, lateral walls covered with such setae and with some longitudinal ridges. Third thoracic sternite convex posteriorly, trilobite anteriorly. Telson 7-plated. Eyes large. Anterior margin of basal segment of antennulae with several spines, usually 5–6, the anterodistal angle produced into a narrow, serrated, spoon-like lobe. First movable segment of antennae with a strongly projecting, serrate-edged, lamellar lobe, bearing 1 or 2 spinules; second segment granular, anterodistal angle with a conical projection; third segment nearly smooth, and with a median tooth-like lobe on terminal margin; flagellum naked. Ischium of third maxilliped broad, rounded, laterodistal edge with a brush of feathered setae (omitted in Fig. 5D); merus triangular, with laterodistal angle usually spine-tipped; carpus with a few longitudinal plications. Chelipeds subequal, ischium with some piliferous plications; merus with transverse piliferous striations, with serrate, spine-tipped lobe on anterior margin, flexor margin with 1 or 2 forward-directed spines towards posterior angle, and an additional one on medial surface of posterior half; ventral surface with either strong or blunt subterminal spine on anterior half; carpus more than twice as long as broad, armed on anterior margin with 3 low, broad, serrate-edged teeth; surface with large, flattened piliferous tubercles, median ones largest and forming a longitudinal row; some tubercles along posterior margin produced into spines (0–9, usually more than 5); distal angle projected into 2 superimposed prominent spines. Manus broad, flattened, covered with tubercles similar to those of carpus, but more rounded; outer margin of manus moderately convex, with a row of tubercles, the most proximal ones usually produced into spines (0–12, usually more than 4); inner margin with row of strongly marked tubercles; outer margin of movable finger with row of strongly marked tubercles. Ventral surface of chelipeds with tubercle-shaped plications; gape of fingers with an extended short, thick pubescence. Walking legs with small, flattened tubercles; anterior margin of merus with a fringe of plumose setae, all segments with irregularly set, long simple setae; anterior margin of merus of leg one with 9–11 spines, of leg two with 10–14, of leg three with 5–11; posterior margin of merus of legs one and two with 1–3 posterodistal spines; propodus with a median spine on ventral surface and a terminal triplet of movable spines on ventral border; ratio between the lengths of carapace and propodus of leg one ranging from 2.13 to 2.60 (average 2.41), of leg two from 1.98 to 2.17 (average 2.04), of leg three from 2.07 to 2.28 (average 2.23); dactylus with 3 movable spinules on inner border.

Colouration. Striations of carapace show dark red spots with orange contours, on a light orangish-brown background; cervical groove light orange; supraocular and epibranchial spines dark orange; all segments of antennae with a light pink, irregular stripe which extends along segments, flanked by broad, dark orange bands along anterior region, and more irregularly along posterior one; serrate lamellar lobe of first movable segment with a light blue spot on a brown to purple-brown background; flagellum brownish purple; flattened tubercles of chelipeds dark orangish red at the tip on a light pink and orange background; outer margin of manus with dark reddish-purple tubercles proximally, corresponding to the spine-bearing ones, and interrupted by three or more orange tubercles; ventral surface of chelipeds reddish purple with some purple and orange spots; gape pubescence orange; ischium, merus and carpus of third maxillipeds dark orangish red; propodus dark orangish red, dactylus purple. Merus of walking legs coloured as carapace; carpus brownish orange with a shimmer of blue, proximally ending in a vivid orange band; approximately first and last quarter of propodus light blue, flanking a broad, vividly orange band; dactylus orange to purple. Walking legs reddish purple in ventral view.

Biology. Our collection data indicate that *P. donadio* occurs from the lower intertidal to 35 m depth, under rocks, among coral rocks, and occasionally underneath *Diadema mexicanum* A. Agassiz. Haig (1960) reported *P. edwardsii* from coral heads to a depth of about 11 m. This finding probably corresponds to *P. donadio* (see discussion). Ovigerous females have been collected in every month from December through February, and in May, August and September.

Geographic range. According to collection data, *P. donadio* ranges from Santa María Bay, Baja California, and the Gulf of California through Central America and the Colombian Pacific, including Malpelo Island, to the southern coast of Ecuador. Further examination of material is needed to complement the geographic range of this species.

Remarks. Haig (1960) reviewed the synonymy of *P. edwardsii.* The species has been frequently confused with similar species from the same morphological group within *Petrolisthes* (e.g. Lockington 1878; Boone 1931). *Petrolisthes agassizii* is morphologically the most closely related species to *P. edwardsii* and *P. donadio.* It can be distinguished by having the cheliped's carpus 3 times as long as wide, with 3 longitudinal rows of piliferous tubercles, and by the carapace striations being

continuous across the metabranchial regions, not interrupted as in *P. edwardsii* and *P. donadio*.

In summary, the differences that distinguish *P. donadio* n. sp. from *P. edwardsii* are: (i) the presence of a supraocular spine (Figs. 1, 4); (ii) the anterior margin of the basal segment of antennula bears several spines (usually 5–6; Fig. 5C), whereas in *P. edwardsii* this margin is spined only proximally, the spines are smaller and blunt, and the anterodistal spoon-shaped angle is broader (Fig. 2C); (iii) the external margin of the cheliped's manus is less convex (Fig. 6A), and the chelipeds are lighter and narrower than in *P. edwardsii* (Fig. 3A); (iv) the propodi of all three walking legs are significantly longer than in *P. edwardsii* (*U*-test, $p < 0.001$), which gives *P. donadio* a more gracile appearance (Figs. 1, 3C–F, 4 and 6C–F); (v) the posterior margin of the cheliped's carpus and the outer margin of the manus tend to bear more spines (Fig. 6A) than in *P. edwardsii* (Fig. 3A); (vi) the two species differ in colour and colour pattern, most conspicuous in *P. donadio* due to the wide, bright orange band on the dorsal side of the propodi of the walking legs; in *P. edwardsii*, approximately the first third of the propodus is light-brown banded, and the remaining two-thirds show a reddish purple band, showing a subterminal, narrow, orange band towards flexor margin.

Intraspecific variation in the number of spines on the posterior margin of the cheliped's carpus, and on the external margin of the cheliped's manus was observed in the two species. They display different trends in respect of these characters, but their ranges overlap. *P. donadio* tends to be spinier in the characters mentioned, with most individuals bearing more than 5 spines on the posterior margin of the carpus, and more than 4 spines on the outer margin of the manus. Contrastingly, in *P. edwardsii* these characters tend to bear fewer spines, with most specimens bearing less than 3 spines on carpus and less than 3 on manus. The *P. donadio* individuals from Cape San Lucas at the mouth of the Gulf of California are the least spined, the spines also appearing mostly blunt. Some *P. edwardsii* specimens from Galapagos display more spines than usual. The spination of these and other characters (e.g. the anterior margin of the merus of the walking legs) may also vary in the same specimen.

Molecular analysis

The COI gene-fragment sequences of 22 specimens included in the molecular dataset were 635 bp long (primers excluded). Their alignment did not generate any gaps and comprised 635 sites, 164 of which were variable. Of these, 107 were parsimony informative. Most variable sites corresponded to third positions (88.41%), followed by first positions (10.98%). Of the parsimony informative sites, 87.85% and 12.15%

Table 2. Averages and ranges of sequence divergence (*p*-distances) at the COI-gene fragment for three species of *Petrolisthes*

| Sequence comparison	*p*-distance	
	Average	Range
Within *P. edwardsii*	0.043	0.002–0.081
Within *P. donadio*	0.017	0.008–0.031
P. edwardsii vs. *P. donadio*	0.120	0.102–0.133
P. edwardsii vs. outgroup	0.151	0.144–0.158
P. donadio vs. outgroup	0.155	0.150–0.160

Outgroup: *P. armatus*.

corresponded to third and first positions, respectively. No identical haplotypes were identified. Sequence divergence (*p*-distances; Table 2) within *P. edwardsii* averaged 0.043. The largest values within this species resulted from comparison among individuals from the Colombian Coast and the individual from Galapagos Islands (average *p*-distance of 0.078), and among these individuals and those from Malpelo Island (average *p*-distance of 0.068). The distance between the individuals from Malpelo and the one from Galapagos was of a similar magnitude (average *p*-distance of 0.063). Contrastingly, divergence within the new species was lower, averaged at 0.017. Distances among the specimens from the Colombian coast and the specimen from Malpelo Island were relatively small (average at 0.014), compared to the distance between the continental *P. edwardsii* individuals and those from Malpelo or Galapagos. Distances between the two species ranged between 0.102 and 0.133, which is greater than the largest distance revealed within each species. Distances between *P. edwardsii* and *P. armatus* (outgroup) ranged from 0.144 to 0.158. The new species and the outgroup diverged with distances between 0.150 and 0.160.

The MP analysis yielded four most parsimonious trees, which were used to obtain the majority-rule consensus tree shown in Fig. 7A. The ML analysis yielded one tree (Fig. 7B), which is similar to the majority-rule consensus tree. The tree obtained through Bayesian inference (not shown) was very similar to the ML tree. The posterior probability values obtained through Bayesian inference are shown at the nodes of the ML tree (Fig. 7B).

Both MP and ML reconstructions support the monophyly of *P. edwardsii* and *P. donadio*. The strong support for each clade is evidenced by large bootstrap and decay-index values (Fig. 7A), and by large posterior probability values (Fig. 7B). The dominance of transitions (Ti) over transversions (Tv) observed in protein-coding genes of animal mtDNA (Moritz and Hillis 1996) was confirmed for our dataset with a Ti:Tv ratio of 5.36. Considering this strong bias towards Ti, which are more
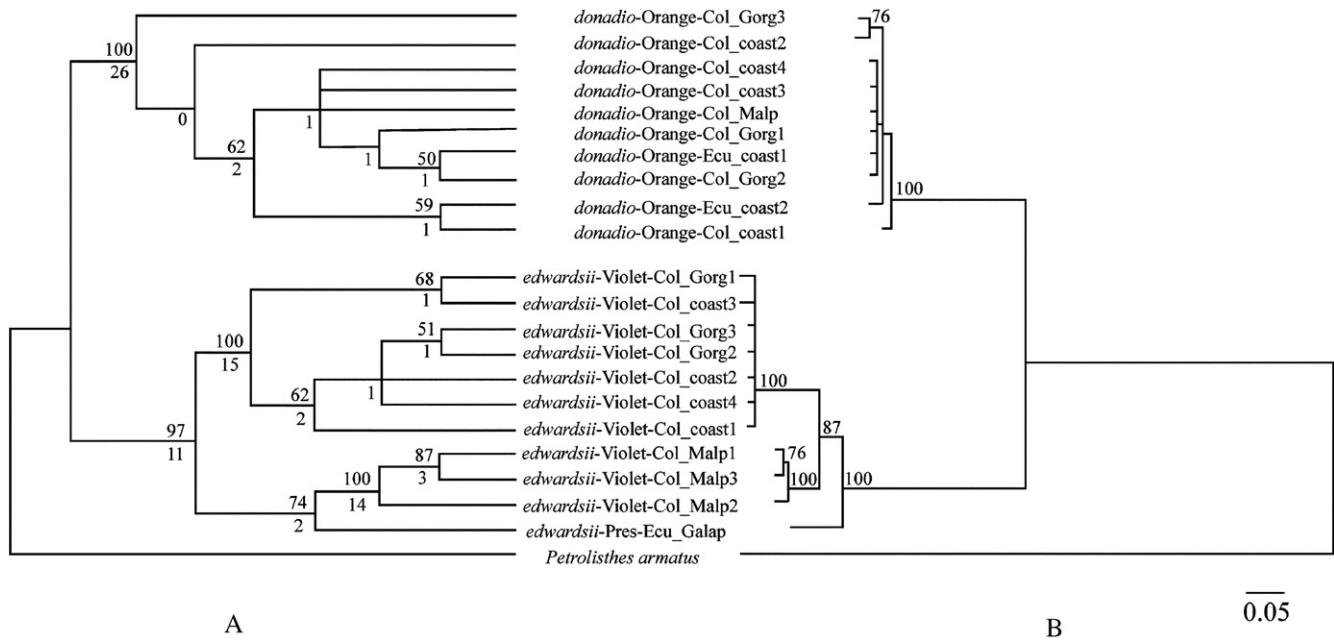
Fig. 7. Phylogenetic trees based on 22 sequences of a fragment of the mitochondrial COI gene, representing *Petrolisthes edwardsii*, *P. donadio* n. sp., and the outgroup *P. armatus*. Sequences are designated as in Table 1, according to the colouration of specimens and their geographic location. (A) Majority-rule consensus of 4 most parsimonious trees ($L = 251$; RI $= 0.9158$); bootstrap values based on 1000 replicates shown above nodes (values higher than 50% only); decay values shown below nodes. (B) Maximum-likelihood tree (–Ln likelihood $= 2047.515$; estimated proportion of invariable sites $= 0.7044$; estimated value of gamma shape parameter $= 418$), obtained after heuristic search (TBR swapping algorithm), using majority-rule parsimony tree to estimate parameters of substitution model, and as starting tree for branch-swapping; values on nodes correspond to posterior probabilities obtained from Bayesian analysis (see text).

prone to homoplasy than Tv (Swofford et al. 1996), we considered only Tv as supporting evidence for clade monophyly. The monophyly of *P. edwardsii* and of *P. donadio* is evidenced by several Tv supporting their respective clades. The *P. edwardsii* clade was supported by five Tv, and the *P. donadio* clade by four.

The COI data show no evidence of phylogeographic subdivision within *P. donadio*. Within *P. edwardsii* some level of phylogeographic structure was evidenced by a clade containing all individuals from Malpelo Island, which was consistently supported in all phylogenetic reconstructions (Figs. 7A and B). This clade showed large support values, as did the group including the remaining individuals except for the one from Galapagos, positioned as a basal lineage to the Malpelo clade in the MP reconstruction, and as basal to the *P. edwardsii* clade in the ML reconstruction.

The likelihood-ratio test of rate constancy resulted in rejection of the molecular clock ($P < 0.05$).

Discussion

Demonstration of genetic differentiation in sympatry constitutes a powerful way of detecting sibling species and can be used to discriminate species-level morpho-logical differences from morphological variation within populations (Palumbi 1997). On the other hand, different species concepts have distinct implications for the use and interpretation of genetic data (Knowlton 2000), making the designation of new species on molecular data a controversial issue. In the present study, concordance between morphological characters and molecular data provides a robust base for the delineation of species boundaries between two sympatric morphotypes recognised for more than a century as *P. edwardsii*. Different approaches to the analysis of COI-sequence variation consistently supported the distinction of two clades or lineages diagnosable through colouration and subtle morphological differences, irrespective of the geographic origin of the specimens. These results give further evidence of the status of each morphotype as a separate species. Therefore, one of the forms is here described as *Petrolisthes donadio* n. sp., the other one is redescribed as *P. edwardsii*.

Within several groups of decapod crustaceans, colour and colour pattern have been reliable characters to distinguish a number of species that differ little in morphology, therefore proving to be of systematic importance (Bruce 1975; Knowlton 1986; Knowlton and Mills 1992; Sarver et al. 1998). The validity of

colour distinctiveness in giving systematic resolution to a complex of species within *Petrolisthes* designated by Werding (1983) as the *P. galathinus* complex, was corroborated by combining molecular, morphological (adult and larval) and geometric morphometric data (Hiller et al. 2006). In both the *P. edwardsii*–*P. donadio*- and the *P. galathinus*-complex cases a link between speciation and colour change could suggest an association between colour distinctiveness and genetic divergence. Specific colouration in sympatric porcellanid siblings may be interpreted as a character involved in intraspecific recognition, or as an unselected by-product of adaptation to different ecological requirements (see Hiller et al. 2006). Because *P. edwardsii* and *P. donadio* seem to occupy similar niches in sympatry, which typically comprise hard substrates offered by superimposed rocks, a role of colouration in intraspecific recognition seems possible. A certain amount of ecological divergence between the two species was detected, including differences in the depth range, as *P. edwardsii* appears to have an intertidal and upper subtidal distribution, while *P. donadio* has been reported from the intertidal down to 30 m depth. Additional and more precise ecological information on both species is needed in order to complement any hypothesis on the evolutionary meaning of their colouration.

Different phylogeographic structures detected in *P. edwardsii* and *P. donadio*, as revealed by restricted sampling, constitute a first but limited approach to the reconstruction of the biogeographic history of these species. The geographic subdivision within the *P. edwardsii* clade corresponds to a distinction of the haplotypes from the Colombian coast (including Gorgona, a continental island about 35 km off this coast) from those located in Malpelo Island (an oceanic island about 500 km off this coast) and Galapagos, which are as well clearly differentiated from each other. Within *P. donadio*, the haplotypes from the Colombian coast and Malpelo did not show any pattern of geographic localisation. Genetic discontinuities in a mtDNA intraspecific phylogeny, detected as several mutational steps among arrays of related genotypes (Avise et al. 1987), may evidence longer-term historical population separations (Avise 1994). A total of 13 transitional changes, 8 of which show a high retention index (RI = 1), support the subdivision of the Malpelo haplotypes within *P. edwardsii*. The haplotype from Galapagos shows 2 uniquely derived Tv, considered as autapomorphies. Inclusion of more individuals of *P. edwardsii* from Galapagos in the analysis will probably show that these mutational changes represent synapomorphies supporting this geographical subdivision. Under the assumption that the degree of phylogeographic concordance across separate genealogies increases with time of separation (Avise 1994), it seems plausible to interpret the differences observed among the

P. edwardsii genotypes from Malpelo and Galapagos as evidence of their longer-term historical separation from the continental genetic pool. In the same way, the lack of mutational steps supporting the distinction of the Malpelo haplotype within *P. donadio* may evidence a more recent colonisation event of this species. Differences in species mobility, considered to exert important influences on patterns of mtDNA phylogeographic structure (Avise 1994), are not considered in this case, as dispersal of porcellanids is confined to the larvae, and their dispersal ability is considered similar in most species (Werding et al. 2003).

Two general geographical modes of speciation can be invoked to explain the contemporary sympatric distribution of sister species. Although sympatric speciation is frequently inferred from the sympatric distribution of sister taxa, the allopatric mode of speciation has been considered as the most conservative model (Mayr 1967; see Losos and Glor 2003). According to peripatric speciation, which constitutes one of the subtypes into which allopatric speciation is subdivided, a new population is founded outside the continuous species range by a single colonist (a fertilised female) or a small founder group, and remains isolated long enough to acquire the genetic basis for reproductive isolation (Mayr and Ashlock 1991). The sympatric distribution of *P. edwardsii* and *P. donadio* could be explained under this speciation model, considering that evolutionary change tends to occur very rapidly in the small populations of marine shore animals that become isolated around oceanic islands (Briggs 1974). Although the two species overlap in most of their coastal geographic ranges, their distribution in oceanic islands seems to differ. According to material examined for this study, *P. edwardsii* has been recorded from Galapagos, Cocos and Malpelo islands, while *P. donadio* apparently occurs on Malpelo Island only. Considering this, it is conceivable that larvae of an ancestral form, widely distributed along the American Pacific coast, colonised these islands and established viable populations, which came into isolation after a barrier arose between the coast and the islands, generated by ocean-current shifts. Apparently, the raising of the Isthmus of Panama affected ocean circulation, though it is not clear how long before the final closure this event could have altered surface currents (Lessios et al. 1999). This is highly speculative, as the reconstruction of any speciation scenario demands a relatively accurate estimation of the timing of species diversification in order to link speciation to geographic barriers generated by dated geological events. This estimation is unfeasible in the current study because the hypothesis of rate constancy was rejected for our dataset, and there is no appropriate porcellanid fossil record. An analysis including sequences of the COI gene from individuals sampled across the complete geographical ranges of *P. edwardsii* and *P. donadio* will

contribute to a better understanding of the biogeographic history of these two species.

Including *P. donadio* n. sp., the eastern Pacific porcellanid fauna now consists of 93 species (see Hiller et al. 2004).

Acknowledgements

Appendix A

Material identified as *P. edwardsii* (de Saussure). Abbreviations: F = female; INV-CRU = Museo de Historia Natural Marina de Colombia - MHNMC - INVEMAR, Santa Marta; Isld. = Island; M = male; pers. coll. = personal collection A. Hiller; for additional museum codes see the material and methods section.

Lectotype

M, ANSP CA4152; MEXICO. In order to enhance the stability of nomenclature, the single specimen in the ANSP syntype series that is lacking a supraocular spine is here designated lectotype of *Porcellana edwardsii* de Saussure, 1853.

Other material

COLOMBIA. Chocó, Arusí; intertidal, under rocks, coll. Exp. Nuquí-INVEMAR, 13–23 September 2003: 1M, INV-CRU 5777; 1F(ov.), INV-CRU 5778; 1F, INV-CRU 5779; 5F(3ov.), SMF 31823; 1M, 8F(3ov.), pers. coll. Valle del Cauca, Bahía Málaga, intertidal, under rocks, coll. J.F. Lazarus, May 2005: 1M, pers. coll. Malpelo Island, 6 m, coll. J. Reyes, 6 m, September 2002: 3 juv., pers. coll.; 12-15m, coll. B. Werding, September 2004: 8M, 2F, pers. coll.

COSTA RICA (Cocos Islands). Chatham Bay, shore, 14 January 1938: 1M, LACM. Wafer Bay, shore, 02 March 1933: 1M, 1F, LACM.

ECUADOR (Galapagos Islands). Archipiélago de Colón, West coast of Isla San Salvador (James), shore, rocky ledges, coll. R/V Velero III, 11 December 1934: 2M, 1F(ov.) (cheliped used for DNA isolation), LACM CR 1934-207.6. Albemarle Isld., N of Tagus Hill, shore, 15 January 1934: 2M, 3F(2ov.), LACM. Charles Isld., Black Beach, shore, 18 January 1934: 3M (1 with Bopyridae), 1F(ov.), LACM. Hood Isld., Osborn I, in Gardner Bay, shore, 31 January 1934: 2M, 1F(ov.), LACM. SE of Cormorant Pt., Charles Isld., shore, 29 January 1933: 1M, 2F(ov.), LACM. Archipiélago de Colón, Isla San Salvador (James), James Bay, shore, rock, sand, coll. R/V Velero III, 12 February 1933: 4M, 2F, LACM CR 1933-073.2. Galapagos 84, A. Cohen, AC-gal-22F: 2F, LACM. Archipiélago de Colón, West coast of Isla San Salvador (James), 0°16′10″S, 90°53′45″, shore, rocky ledges, coll. R/V Velero III, Sta. No. 333-35, 11 December 1934: 1M, 2F, LACM CR 1934-207.6. Archipiélago de Colón, Isla San Salvador (James), James Bay, 0°11′54″S, 90°51′20″W, shore, rock, sand, coll. R/V Velero III, Sta. no. 71–33, 12 February 1933: 3M, LACM CR 1933-073.2.

MEXICO. Lower California, Cape San Lucas, coll. John Xantus, from Smithsonian Institute (USNM 1148): 38M (1 with Rhizocephala), 57F(3ov.), MCZ 1370. Gulf of California, Los Frailes, shore, 13 March 1949: 3M, 1F, LACM. Gulf of California, Cape San Lucas, 01 February 1955, shore, K-140, J. Knudsen: 1F, LACM. Gulf of California, March 1956, J. Knudsen: 1M, LACM. Sonora, Isla San Pedro Nolasco, 7 ft, rocks, M. Gilligan, Sta No. UA74-57: 1F(ov.), LACM. Tres Marías Islands, lagoon behind NE Pt. Cleopha Isld., shore, 07 February 1954: 1M, 2F, LACM.

Guerrero, near Acapulco, Puerto del Marqués, shore, Sta No. 1562-46, 15 September 1946: 1M, LACM. Guerrero, Acapulco, shore, granite boulders, coll. R.C. Brusca, 12 June 1979: 1F(ov.), LACM.

PERU. Southern Cancas (ca. 4°00′S), rocky shore, coll. Koepcke, 10 September 1950: 1 dry-preserved cheliped, SMF.

Appendix B

Material identified as *P. donadio* n. sp.

Abbreviations: CL = carapace length; CW = carapace width; F = female; INV-CRU = Museo de Historia Natural Marina de Colombia - MHNMC - INVEMAR, Santa Marta; M = male; pers. coll. = personal collection A. Hiller; for additional museum codes see the material and methods section.

Holotype

F(ov.), INV-CRU 5780; COLOMBIA, Chocó, Arusí, intertidal, under rocks, coll. Exp. Nuquí-INVEMAR, 13–23 September 2003.

Paratypes

COLOMBIA. Same data as holotype: 2M, 3F(1ov.), INV-CRU 5781. Gorgona Island, El Horno, underneath *D. mexicanum*, coll. Schoppe, 02 October 1991: 1F, INV-CRU 5782; Punta Sur, 4 m, coll. Werding, May 1978: 1M, 5F(ov.), pers. coll.; Punta del Cocal, 7–15 m, coll. Werding, May 1979: 1F(ov.), pers. coll. Valle del Cauca, Bahía Málaga, Isla Palma, under rocks, 4 m, coll. Werding, September 2004: 1F, pers. coll.

ECUADOR. Salinas, under rocks, 1–2 m, coll. Werding, 06 August 1993: 1M, 1F(ov.), SMF-31824; under rocks, with sponges, 20–30 m, coll. Werding, August 1993: 2M, SMF-31825.

Other material

COLOMBIA. Same data as holotype: 2F(ov.), pers. coll. Cupica, amongst coral blocks, 4 m, leg. E.M. Poulson, 29 April 1948: 1M, ZMUC-CRU-4867. Port Utría, shore, 24 January 1935: 1M, LACM; 25 February 1938: 1F(ov.), LACM. Octavia Bay, shore, 28 January 1935: 1M, 1F(ov.), LACM. Gorgona Island, La Camaronera, 4–6 m, coll. Werding, May 1979, Cat. no. 1987-02: 1M, 2F(1ov.), LACM; Punta Sur, 4 m, coll. Werding, May 1978: 1M, 3F(ov.), LACM; El Horno, underneath *D. mexicanum*, coll. Schoppe, 02 October 91: 7M, 10F(7ov.), pers. coll.; 15 m, coll. Werding, May

1979: 3M, pers. coll.; Punta del Cocal, 15 m, coll. Werding, May 1979: 3M, 3F(2ov.), pers. coll.; Roca Gorgonilla, 8–12 m, coll. Werding, May 1979: 1M, 4F, pers. coll. Gorgona Island, shore, 12 February 1934: 1F, LACM. Malpelo Island, Arrecife, 12–15 m, coll. Werding, September 2004: 1M, pers. coll.

COSTA RICA. Playa Blanca, shore, 08 February 1935: 3M, 3F(ov.), LACM. Port Parker, shore, 09 February 1935: 1M, 1F(ov.), LACM. Guanacoste Prov., Playa de Tamarindo, coll. R. Casebeer, Acc. No. 1963-12, 28 January 1963: 1M, LACM.

MEXICO. Baja California Sur, Punta Márquez, R. Cimberg, February 1979: 1F, LACM. Lower California, Punta Los Frailes, D.G Lindquist, 2 August 1974: 1M (with Rhizocephala), LACM; Sta. María Bay, shore, March 21, 1933: 1F (with Rhizocephala), LACM; Cape St. Lucas, coll. John Xantus, from Smithsonian Institute: 17M (3 with Rhizocephala), 22F(3ov. and 4 with Rhizocephala), MCZ 1370. Gulf of California, Sonora, Isla San Pedro Nolasco, 30 ft, rocks, Alex Kerstitch, 23 December 1978: 1F(ov.), LACM; South of Punta Arena, 02 February 1971, RV Searcher: 8M (4 with Rhizocephala), 3F (with Rhizocephala), LACM. Isla Isabel, KW.28, shore, J. Knudsen, 22 March 1956: 1M, LACM. Tres Marías Islands, María Magdalena Island, 13 fms, 09 May 1939: 4M, 2F(ov.), LACM. Guerrero, Acapulco, shore, Dawson 123, 02 February 1947: 1M, LACM. Gulf of California or México, 1946 or 1947: 1M, LACM. Paralectotypes of *Porcellana edwardsii* de Saussure, 1853 (see the introduction section): 2F, ANSP CA4152 (the two specimens showing a supraocular spine).

PANAMA. Panama City, Albert Einstein Institute, shore, coll. L.G. Abele, 17 February 1969: 1F, LACM. Taboga Island, shore, low water, October 1915, Dr. Th. Mortensen's Pacific Expedition 1914–1916: 1M, 2F(1ov.), ZMUC-CRU-4865; rocky shore, 21 November 1915, Dr. Th. Mortensen's Pacific Expedition 1914–1916: 1M, ZMUC-CRU-4866. Taboguilla Island, 2 m: 1F, LACM. Secas Islands, coll. P. Castro, 18 August 1974: 3M, 3F(1ov.), LACM; shore, 05–06 Feb. 1935: 2F(1ov.), LACM; shore, 02 March 1938: 3M, 1F, LACM. Piñas Bay, 2–4fms, 29 January 1935: 1M, 1F(ov.), LACM. Perlas Islands, Southwest of the south point of Rey Island, 15fms, dredged muddy sand, 27 January 1916, Dr. Th. Mortensen's Pacific Expedition 1914–1916: 1M, 1F, ZMUC-CRU-4869; Contadora Island, stones, 10fms, 28 January 1916, Dr. Th. Mortensen's Pacific Expedition 1914–1916: 1 juv., ZMUC-CRU-4868.

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