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Decapod Crustacean Phylogenetics

edited by

Joel W. Martin, Keith A. Crandall, and Darryl L. Felder



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Edited by

Joel W. Martin

Natural History Museum of L. A. County
Los Angeles, California, U. S. A.

Keith A. Crandall

Brigham Young University
Provo, Utah, U. S. A.

Darryl L. Felder

University of Louisiana
Lafayette, Louisiana, U. S. A.



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FERNANDO L. MANTELATTO¹, RAFAEL ROBLES², CHRISTOPH D. SCHUBART³
& DARRYL L. FELDER²

¹ Laboratory of Bioecology and Crustacean Systematics, Department of Biology, FFCLRP, University of São Paulo, Brazil

² Department of Biology, Laboratory for Crustacean Research, University of Louisiana at Lafayette, Lafayette, Louisiana, U.S.A.

³ Biologie I, Universität Regensburg, 93040 Regensburg, Germany

ABSTRACT

As currently recognized by most taxonomists, the genus *Cronius* Stimpson, 1860, encompasses only two species, both distributed in tropical and subtropical waters. *Cronius ruber* (Lamarck, 1818) is reported from both the Pacific and Atlantic American coastlines, as well as the eastern Atlantic, and *C. tumidulus* (Stimpson, 1871) is reported to occur exclusively in the tropical western Atlantic. We examine potential differences between allopatric populations assigned to *C. ruber*, test hypothesized monophyly of the genus, and resolve the phylogenetic position of its members within the Portunidae. In so doing, we also revisit taxonomic classification of American species currently assigned to the genus *Portunus*. New 16S mtDNA sequences were obtained from representatives of the genera *Charybdis*, *Cronius*, *Lupella*, *Lupocyclus*, *Polybius*, *Portunus*, and *Thalamita* for examination along with sequences from GenBank. Slight but consistent genetic differences were found among populations assigned to *Cronius ruber* from the Pacific American coastline, the Atlantic American coastline, and the eastern Atlantic coastline (West Africa). The name *C. edwardsii* (Lockington, 1877) is resurrected for specimens from the eastern Pacific, but further analyses are needed to determine if additional taxonomic revisions may be required to more narrowly restrict use of the name *C. ruber* among a complex of Atlantic populations. Presently assigned members of *Cronius* do not form a monophyletic group. The well-defined clade representing *C. ruber* (including the resurrected *C. edwardsii*) is placed in a weakly supported grouping with representatives of *Laleonectes*, *Thalamita*, and *Charybdis*. In contrast, *Cronius tumidulus* forms a well-supported cluster with several present American representatives of the genus *Portunus*, which themselves are well separated from *P. pelagicus*, type species of that genus. Thus, we propose a revised taxonomy with placement of *C. tumidulus* in the resurrected genus *Achelous* De Haan 1833, an assignment that we also propose for nine American species currently treated under *Portunus*.

1 INTRODUCTION

Portunoidea Rafinesque, 1815, *sensu* Martin & Davis (2001) is a highly diverse group that consists of three families: Geryonidae Colosi, 1923, Trichodactylidae H. Milne Edwards, 1853, and Portunidae Rafinesque, 1815. In the latter family, the subfamily Portuninae is the most diverse, containing 11 genera and more than 130 species. While this diverse group of marine and non-marine species shares clearly portunid adaptations, evolutionary lineages among the genera are poorly understood. Despite numerous studies on its classification (see Karasawa et al. 2008 for review), Portunidae is one of a few brachyuran families that have undergone little taxonomic revision in recent years. Systematic review is warranted to reflect current evidence of phylogenetic relationships among its constituent genera.

The genus *Cronius* was described by Stimpson (1860), being based upon “the *Lupa rubra* [= *Portunus ruber*] of M. Edwards, which forms the connecting link between the old genus *Lupa*, and *Charybdis*.” Under current systematic treatments, the two species assigned to this genus are *Cronius ruber* (Lamarck, 1818) and *C. tumidulus* (Stimpson, 1871) (originally as *Acheloius tumidulus*). However, another two species were once proposed but later synonymized. These are *C. millerii* (A. Milne-Edwards, 1868) from East Africa, which most authors consider a synonym of *C. ruber* (e.g., Rathbun 1930 and as discussed in Manning & Holthuis 1981), and *C. edwardsii* (Lockington, 1877) from the eastern Pacific.

The “blackpoint sculling crab” *Cronius ruber* is a typically shallow water species found among a variety of substrates, especially rock rubble in the sublittoral areas (including tide pools), but there are a few reports to depths near 100 m. Its reported distribution extends from New Jersey (USA) throughout the Gulf of Mexico and the Caribbean to Rio Grande do Sul (Brazil) in the western Atlantic; from California to Peru and the Galapagos Islands in the eastern Pacific (if accepting *C. edwardsii* as a synonym); and from Senegal to Angola along the eastern Atlantic (if accepting *C. millerii* as a synonym). However, recent translocation and rapid expansion of *Charybdis hellerii* (A. Milne-Edwards, 1867) into the western Atlantic (see Mantelatto & Dias 1999 for review), a species that also thrives in shallow rocky areas, seems to have a negative impact on native species (Mantelatto & Garcia 2001), and sympatric populations of *C. ruber* appear to be in decline along the Brazilian coast (FLM, personal observation). In contrast, the “crevice sculling crab” *C. tumidulus* is primarily resident on open areas of shallow waters, including seagrass bottoms, back-reef coral heads and flats, and coral reefs (FLM and DLF, personal observations). This species is found only in the western Atlantic and is currently reported only from Bermuda and Florida to Brazil (Rathbun 1930; Williams 1984; Melo 1996).

It is noteworthy that almost 150 years ago Stimpson (1860) considered *Cronius ruber* to potentially represent a link between *Portunus* Weber, 1795, and *Charybdis* De Haan, 1833. Given this potentially unique but uncertain phylogenetic position for *Cronius*, it was essential for us to include selected members of the subfamilies Portuninae and Thalamitinae in our analyses in order to test monophyly of the genus as well as its phylogenetic position within the Portunidae. At the same time, phylogeny and taxonomy of the widely distributed genus *Portunus* has long been a topic of debate (e.g., Stephenson & Campbell 1959), and polyphyly of the genus *Portunus* has been clearly demonstrated by Mantelatto et al. (2007). In this recently published molecular phylogeny, only the species *P. sayi* (Gibbes, 1850), among all included western Atlantic representatives of the genus, clustered with the Indo-West Pacific type species of the genus, *P. pelagicus* (Linnaeus, 1758). This lineage grouped with *Callinectes* Stimpson, 1860, and *Arenaeus* Dana, 1851, instead of other included species of *Portunus*. The other western Atlantic representatives of *Portunus* and *Laeonectes vocans* (A. Milne-Edwards, 1878) were instead consistently separated from this group and thus were noted to warrant eventual reclassification.

The current study aims to build on the molecular phylogeny of Mantelatto et al. (2007) by use of the same genetic marker, 16S mtDNA, but with inclusion of additional taxa representing the Portuninae and Thalamitinae. Special emphasis is given to the genus *Cronius* and constituent species

in order to: 1) test intraspecific variability within *C. ruber* and the possible validity of *C. millerii* and *C. edwardsii*; 2) test monophyly of the genus *Cronius*; and 3) test the position of *Cronius* within the Portuninae and its postulated link to the subfamily Thalamitinae. On the basis of these results, we propose taxonomic reclassifications for the species and genera under study.

2 MATERIALS AND METHODS

2.1 Sample collection

Portunid crabs used in this study were newly collected or obtained as gifts or loans from museum collections (Table 1). Newly collected specimens for DNA analysis were preserved directly in 75 to 90% ethanol. Species identifications were confirmed on the basis of morphological characters from available references (Stimpson 1860; Rathbun 1930; Stephenson & Campbell 1959; Manning & Holthuis 1981; Williams 1984; Manning & Chace 1990). Voucher specimens from which tissue subsamples were taken have been deposited in permanent collections (Table 1). Tissues from paratype and holotype materials, excised by minimally destructive methods, were sequenced when possible (Table 1).

Along with populations of *Cronius* from both sides of the Atlantic Ocean and the eastern Pacific Ocean, we included several species representing *Portunus* and other genera of the family Portunidae for comparison, initially to more broadly root the analysis. It was essential to include other members of the subfamilies Portuninae and Thalamitinae in order to test monophyly of the genus *Cronius* and to determine its phylogenetic position within the Portunidae. Specifically, we used all sequences of 12 species of *Portunus* from the western Atlantic attained in the previous study on molecular phylogeny by Mantelatto et al. (2007); additional species of *Portunus* from the eastern Pacific (Mexico), eastern Atlantic (Mediterranean), and Indo-West Pacific; *Charybdis* from the Atlantic, and Indo-West Pacific; *Euphylax* Stimpson, 1860, from the eastern Pacific (Mexico); *Laeonectes* Manning & Chace, 1990, from the Atlantic; and species of *Lupocyclus* Adams & White, 1848, and *Thalamita* Latreille, 1829, from the Indo-Pacific. Additionally, specimens of the portunid crab genera *Ovalipes* Rathbun, 1898, and *Polybius* Leach, 1820, (Polybiinae) and *Carcinus* Leach, 1814, (Carcininae) were included in the analysis as outgroups because they putatively represented successively more distant lineages from the in-group taxa. Some of the comparative sequences included in the analysis were retrieved from GenBank (Table 1).

2.2 DNA analysis

We based our phylogenetic analysis exclusively on a partial fragment of the 16S rDNA gene, which has repeatedly shown its utility in both phylogenetic and population studies for more than a decade and is thus a common choice for use in phylogenetic studies on decapods (see Schubart et al. 2000 and Mantelatto et al. 2007 for literature review). DNA extraction, amplification, and sequencing protocols were implemented as per Schubart et al. (2000) with modifications as in Mantelatto et al. (2007) and Robles et al. (2007).

Total genomic DNA was extracted from muscle tissue of walking legs or chelipeds. Muscle was ground and incubated for 1–12 h in 600 μ l lysis buffer at 65°C; protein was separated by addition of 200 μ l 7.5 M ammonium acetate prior to centrifugation. DNA precipitation was made by addition of 600 μ l cold isopropanol followed by centrifugation; the resultant pellet was washed with 70% ethanol, dried, and resuspended in 10–20 μ l TE buffer.

An approximately 560-basepair region of the 16S rRNA gene was amplified from diluted DNA by means of polymerase chain reaction (PCR) (thermal cycles: initial denaturation for 10 min at 94°C; annealing for 38–42 cycles: 1 min at 94°C, 1 min at 45–48°C, 2 min at 72°C; final extension of 10 min at 72°C) with the following primers: 16Sar (5'-CGC CTG TTT ATC AAA AAC AT-3'), 16Sbr (5'-CCG GTC TGA ACT CAG ATC ACG T-3'), 16SH4 (5'-GTY GCC CCA ACC AAA

Table 1. Portunid crab species used for phylogenetic reconstructions, showing respective date and site of collection along with museum catalog number (ULLZ: University of Louisiana—Lafayette Zoological Collections; IVIC: Instituto Venezolano de Investigaciones Científicas—Laboratorio de Ecología y Genética de Poblaciones, Crustacean Collection “Dr. Gilberto Rodríguez,” CCDB: Crustacean Collection of Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo; USNM: National Museum of Natural History, Smithsonian Institution, Washington D.C.; SMF: Senckenberg Forschungsinstitut und Museum, Frankfurt; MNHN: Muséum National d’Histoire Naturelle, Paris; CSIRO: Marine Research, Invertebrate Museum, Hobart) and GenBank accession number.

Species	Collection site, date	Catalogue No.	GenBank accession number
<i>Arenaeus cribrarius</i> (Lamarck, 1818)	Venezuela: Falcón, 1999	ULLZ 5173	DQ407667 ^c
<i>Callinectes bellicosus</i> Stimpson, 1859	Mexico: Baja California, 1999	ULLZ 4166	DQ407670
<i>Callinectes bocourti</i> A. Milne-Edwards, 1879	Venezuela: Zulía, 1999	ULLZ 4180	AJ298170
<i>Callinectes danae</i> Smith, 1869	Venezuela: Falcón, 1998	IVIC-LEGP-C-1	AJ298184 ^a
<i>Callinectes ornatus</i> Ordway, 1863	Brazil: São Paulo, 1999	ULLZ 4178	AJ298186 ^a
<i>Callinectes sapidus</i> Rathbun, 1896	USA: Florida, 1998	ULLZ 3766	AJ298189
<i>Carcinus maenas</i> Linnaeus, 1758	USA: New Hampshire, 1998	ULLZ 3840	AJ130811
<i>Charybdis hellerii</i> (A. Milne-Edwards, 1867)	Brazil: São Paulo, 1995	CCDB 2038	FJ152142
<i>Charybdis feriatius</i> (Linnaeus, 1758)	China, 2005	—	DQ062727
<i>Cronius ruber</i> (Lamarck, 1818)	Ghana: Cape Coast, 2001	SMF 31986	FJ153143
<i>Cronius ruber</i> (Lamarck, 1818)	Mexico: Veracruz, 2002	ULLZ 6448	FJ152144
<i>Cronius ruber</i> (Lamarck, 1818)	Brazil: São Paulo, 1999	ULLZ 4295: CCDB 138	FJ152145
<i>Cronius ruber</i> (Lamarck, 1818)	Brazil: São Paulo, 2000	ULLZ 4772	FJ152146
“ <i>Cronius ruber</i> ” (Lamarck, 1818) ^{***}	Panama: Pacific coast, 2007	ULLZ 8673	FJ152147
“ <i>Cronius ruber</i> ” (Lamarck, 1818)	Panama: Pacific, Gulf of Chiriqui, 2007	CCDB 1717	FJ152148
“ <i>Cronius tumidulus</i> ” (Stimpson, 1871)	Brazil: Ubatuba, 2000	ULLZ 4770	FJ152149
“ <i>Cronius tumidulus</i> ” (Stimpson, 1871)	USA: Gulf of Mexico, 2005	ULLZ 6838	FJ152150
“ <i>Cronius tumidulus</i> ” (Stimpson, 1871)	Providencia, Colombia, Caribbean, 1998	ULLZ 9117	FJ152151
“ <i>Cronius tumidulus</i> ” (Stimpson, 1871)	Puerto Rico: Paguera, 1995	USNM uncatalogued	FJ152152
<i>Euphyllax robustus</i> A. Milne-Edwards, 1874	Costa Rica: Gulf of Nicoya, 2004	CCDB 1122	FJ152153
<i>Laeonectes nipponensis</i> (Sakai, 1938)	French Polynesia, no date	MNHN-B 31434	FJ152154
<i>Laeonectes vocans</i> (A. Milne-Edwards, 1878)	USA: Louisiana, 2000	ULLZ 4640	DQ388051 ^d
<i>Lupella forceps</i> (Fabricius, 1793)	R/V Oregon II, 1970	USNM 284565	FJ152155
<i>Lupocyclus philippinensis</i> Semper, 1880	China, 1998	—	FJ152156
<i>Ovalipes stephensoni</i> Williams, 1976	USA: Florida, 2003	ULLZ 5678	DQ388050 ^d
<i>Ovalipes trimaculatus</i> (De Haan, 1833)	Argentina: Mar del Plata, 2001	ULLZ 4773	DQ388049 ^d
<i>Polybius henslowii</i> Leach, 1820	Spain: Santander, 1992	SMF 31991	FJ152157
<i>Portunus anceps</i> (Saussure, 1858)	Belize: Carrie Bow Cay, 1983	ULLZ 4327	DQ388054 ^d
“ <i>Portunus asper</i> ” (A. Milne-Edwards, 1861)	Mexico: Sinaloa, 2004	CCDB 1738	FJ152158
“ <i>Portunus binocularis</i> ” Holthuis, 1969 ^{**}	USA: NW Atlantic, 1965	USNM 113560	DQ388062 ^d
“ <i>Portunus depressifrons</i> ” (Stimpson, 1859) [*]	USA: Florida, 1996	ULLZ 4442	DQ388064 ^d
<i>Portunus floridanus</i> Rathbun, 1930	USA: Gulf of Mexico, 2000	ULLZ 4695	DQ388058 ^d
“ <i>Portunus gibbesii</i> ” (Stimpson, 1859)	USA: Alabama, 2001	ULLZ 4565	DQ388057 ^d
<i>Portunus hastatus</i> (Linnaeus, 1767)	Turkey: Beldibi, 2007	SMF 31989	FJ152159
“ <i>Portunus ordwayi</i> ” (Stimpson, 1860) ^{**}	USA: Florida, 1915	USNM 61174	DQ388066 ^d
“ <i>Portunus ordwayi</i> ” (Stimpson, 1860)	Jamaica: St. Ann – Priory, 2003	SMF 31988	FJ152160
<i>Portunus pelagicus</i> (Linnaeus, 1758)	China, 2005	—	DQ062734
<i>Portunus pelagicus</i> (Linnaeus, 1758)	India: Gulf of Mainnar, 2003	ULLZ 5682	DQ388052 ^d
<i>Portunus pelagicus</i> (Linnaeus, 1758)	Australia: Tasmania, no date	CSIRO uncatalogued	FJ152161
“ <i>Portunus rufiremus</i> ” Holthuis, 1959 ^{**}	French Guiana: Sinnamaryi, 1974	USNM 151568	DQ388063 ^d
<i>Portunus sayi</i> (Gibbes, 1850)	USA: Louisiana, 2001	ULLZ 4753	DQ388053 ^d
“ <i>Portunus sebae</i> ” (H. Milne Edwards, 1834)	USA: Florida, 2001	ULLZ 4527	DQ388067 ^d
“ <i>Portunus spinicarpus</i> ” (Stimpson, 1871)	USA: Florida, 1996	ULLZ 4618	DQ388061 ^d
“ <i>Portunus spinimanus</i> ” Latreille, 1819	Jamaica: St. Ann – Priory, 2003	SMF 31987	FJ152162

Table 1. continued.

Species	Collection site, date	Catalogue No.	GenBank accession number
<i>Portunus trituberculatus</i> (Miers, 1876)	Japan, 2002	—	AB093006
<i>Portunus ventralis</i> (A. Milne-Edwards, 1879)	Belize: Carrie Bow Cay, 1983	ULLZ 4440	DQ388060 ^d
<i>Scylla olivacea</i> (Herbst, 1796)	Taiwan, 2003	—	AF109321 ^b
<i>Scylla paramamosain</i> Estampador, 1949	Taiwan, 1998	—	AF109319
<i>Scylla serrata</i> (Forskål, 1775)	Taiwan, 2003	—	AF109318 ^b
<i>Scylla tranquebarica</i> (Fabricius, 1798)	Taiwan, 1998	—	AF109320
<i>Thalamita admete</i> Herbst, 1803	South Africa, 2001	ULLZ 4382	FJ152163
<i>Thalamita crenata</i> Latreille, 1829	Hawaii, Oahu, 2003	ULLZ 8664	FJ152164
<i>Thalamita danae</i> Stimpson, 1858	Singapore: Labrador, 1999	ULLZ 4760	FJ152165
<i>Thalamita sima</i> H. Milne Edwards, 1834	Australia, 1980	ULLZ 4761	FJ152166

Specimens used for DNA analysis: * type; ** holotype.

^aSchubart et al. 2001b; ^bHideyuki et al. 2004; ^cRobles et al. 2007; ^dMantelatto et al. 2007.

***Quote marks (“ ”) are used to show commonly used present names that are proposed for revision in this paper.

TAA A-3'), 16SL2 (5'-TGC CTG TTT ATC AAA AAC AT-3'), 16SH2 (5'-AGA TAG AAA CCA ACC TGG-3'), 16SL15 (5'-GAC GATA AGA CCC TAT AAA GCT T-3') (for references on the primers, see Schubart et al. 2000 and Schubart et al. 2001a). We used 16SH4 and 16SL15 internal primers (in combination with 16SL2, 16Sar, and 16Sbr) for partial amplification of the possibly formalin-fixed specimens among museum materials. PCR products were purified using Microcon 100[®] filters (Millipore Corp.) and sequenced with the ABI PRISM[®] Big Dye[™] Terminator Mix (Applied Biosystems) in an ABI PRISM[®] 3100 Genetic Analyzer (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands.

2.3 Phylogenetic analyses

A consensus sequence for the two strands was obtained and multiple alignments were performed using the Clustal W option as implemented in the sequence alignment editor BioEdit ver. 7 (Hall 1999). Phylogenetic and molecular evolutionary analyses were conducted using MRBAYES software for Bayesian analysis (BAY) and PAUP 4.0 b10 (Swofford 2000) for the maximum parsimony (MP) and neighbor joining (NJ) analyses. Sequences were first analyzed with the software MODELTEST (Posada & Crandall 1998) in order to find the model of evolution that best fit the data. The BAY analysis was performed sampling 1 tree every 500 generations for 2,000,000 generations, starting with a random tree using the model of evolution obtained with MODELTEST, thus obtaining 4,001 trees. Preliminary analysis showed that stasis was reached at approximately 25,000 generations; we discarded the first 30,000 generations and the initial random tree (= 61 trees) and obtained a majority rule consensus tree from the remaining 3,940 trees. NJ analysis was carried out with a maximum likelihood distance correction set, with the parameters obtained by MODELTEST. MP analysis was performed as a heuristic search with random sequence addition of 1000 random trees, including tree bisection and reconnection as a branch swapping option; ten trees were saved after every repetition; indels were treated as a fifth character. On molecular trees, bootstrap confidence values >50% were reported for both NJ (2000 bootstraps) and MP (2000 bootstraps). For the BAY analysis, values were shown for posterior probabilities of the nodes among the 3,940 saved trees. Sequences, as well as the complete alignment, have been deposited in GenBank (Table 1).

3 RESULTS

3.1 Taxonomic account

Morphological data, historical synonymies, and diagnoses for both species of *Cronius* have been gathered from descriptions in the references mentioned in the introduction, especially Stimpson

(1860), Rathbun (1930), Garth & Stephenson (1966), Manning & Holthuis (1981), and Williams (1984). Morphologically, we limited review of the literature and our materials to a search for diagnostic differences that might support our molecularly based hypotheses. Specimens examined are those deposited in the collections mentioned in Table 1. CW = carapace width, measured between the penultimate and posteriormost lateral spines.

Cronius ruber (Lamarck, 1818) *sensu lato*

Material examined: 1 ♂ (81.77 mm CW), 2 ♀ (70.21, 78.95 mm CW), Brazil, São Paulo, Ubatuba, July 1998, CCDB 1445; 1 ♂ (82.81 mm CW), Brazil, São Paulo, Ubatuba, Ilha Anchieta, July 1999, ULLZ 4295 (only pereopods 4 and 5 as DNA voucher CDS) and CCDB 138; 1 ♂ (44.0 mm CW), Brazil, São Paulo, Ubatuba, Ilha Anchieta, June 2000, ULLZ 4772 (DNA voucher FLM); 1 ♂ (6.7 mm CW), Mexico, SW Gulf of Mexico, June 2005, ULLZ 7352; 1 ♀ (6.5 mm CW), USA, off Louisiana, Gulf of Mexico, June 2006, ULLZ 8180; 2 ♂ (43.2, 46.7 mm CW), 1 ♀ (50.5 mm CW), USA, Newfound Harbor Keys, Florida, June 1979, ULLZ 2288; 1 ♀ (50.1 mm CW), USA, Port Mansfield, Texas, August 1969, ULLZ 8662; 1 juvenile ♂ (7.6 mm CW), Mexico, Veracruz, Laguna La Mancha, July 2002, ULLZ 6448; 3 ♀ (18.3, 28.5, 33.4 mm CW), Mexico, Baja California, Isla del Carmen, January 1932, USNM 207834; 1 ♂ (17.4 mm CW), Panama, Pacific coast, 9 May 2005, CCDB 1717; 1 ♂ (14.6 mm CW), Panama, Pacific coast, 15 February 2007, ULLZ 8673; 1 ♂ (53.2 mm CW), Ecuador, September 1926, USNM 76854; 1 ♂ (23.7 mm CW), Venezuela, Cariaco Basin, NW of Barcelona, October 1963, USNM 152578; 1 ♀ (38.9 mm CW), Saint Lucia, Caribbean Sea, E of Saint Lucia, March 1966, USNM 180526; 1 ♂ (20.8 mm CW), 1 ♀ ovigerous (42.3 mm CW), USA, off Florida, Gulf of Mexico, SOFLA expedition, April 1981, USNM 242921; additional material examined labeled as *Cronius millerii* (A. Milne-Edwards, 1868): 1 ♂ (73.4 mm CW), 1 ♀ (71.8 mm CW); Ghana: Cape Coast, July 2001 (both DNA vouchers); 1 ♂ (not measurable mm CW), Senegal, Dakar, November 1950, USNM 173088.

Cronius tumidulus (Stimpson, 1871)

Material examined: 1 ♂ (24.5 mm CW), USA, Florida, Tortugas Isl., July 1924, USNM 61015; 1 ♂ (10.5 mm CW), 2 ♀ (8.80, 11.50 mm CW), USA, Florida, off Palm Beach, 1951, USNM 168055; 1 ♀ (11.40 mm CW), USA, Florida, off Palm Beach, April 1950, USNM 169257; 1 ♀ (26.3 mm CW), USA, Puerto Rico, Paguera, Lauri Reef, March 1995, USNM uncatalogued (DNA voucher); 1 ♂ (14.77 mm CW), 2 ♀ (11.62, 10.31 mm CW), Brazil, São Paulo, Ubatuba, February 1999, CCDB 2036; 1 ♀ (10.88 mm CW), Brazil, São Paulo, Ubatuba, March 1996, CCDB 131; 5 ♂ (19.65, 17.40, 15.26, 14.34, 8.82 mm CW), 2 ♀ (17.4, 13.54 mm CW), Brazil, São Paulo, Ubatuba, February 2000, CCDB 128; 1 ♂ (11.36 mm CW), Brazil, São Paulo, Ubatuba, February 1996, CCDB 127; 1 ♂ (15.01 mm CW), Brazil, São Paulo, Ubatuba, February 2000, ULLZ 4770 (DNA voucher FLM); 1 ♀ ovigerous (18.2 mm CW), Mexico, Gulf of Mexico, June 2005, ULLZ 6838; 1 ♀ (6.10 mm CW), Brazil, São Paulo, Ubatuba, Ubatumirim, February 2000, CCDB 2035.

3.2 Molecular phylogeny

In total, 545 positions of the 16S rRNA gene (not including primer regions) were aligned for 49 por-tunid species. The optimal model of evolution for the data set, selected under the Akaike information criterion (AIC) as implemented in Modeltest (Posada & Crandall 1998), was the TVM+I+G (Invariable sites + Gamma distribution) with the following parameters: assumed nucleotide frequencies A = 0.3821, C = 0.0820, G = 0.1446, T = 0.3913; substitution model A-C = 0.8814, A-G = 8.1643, A-T = 1.0082, C-G = 1.0959, C-T = 8.1643, G-T = 1.00; proportion of invariable sites I = 0.2746; variable sites follow a gamma distribution with shape parameter = 0.5018. Thus, posterior analyses are based on this evolutionary model.

The molecular tree (Figure 1) is based on three different algorithms (NJ, MP, BAY), which are mostly congruent. The resultant molecular phylogeny disagrees in several respects with the current

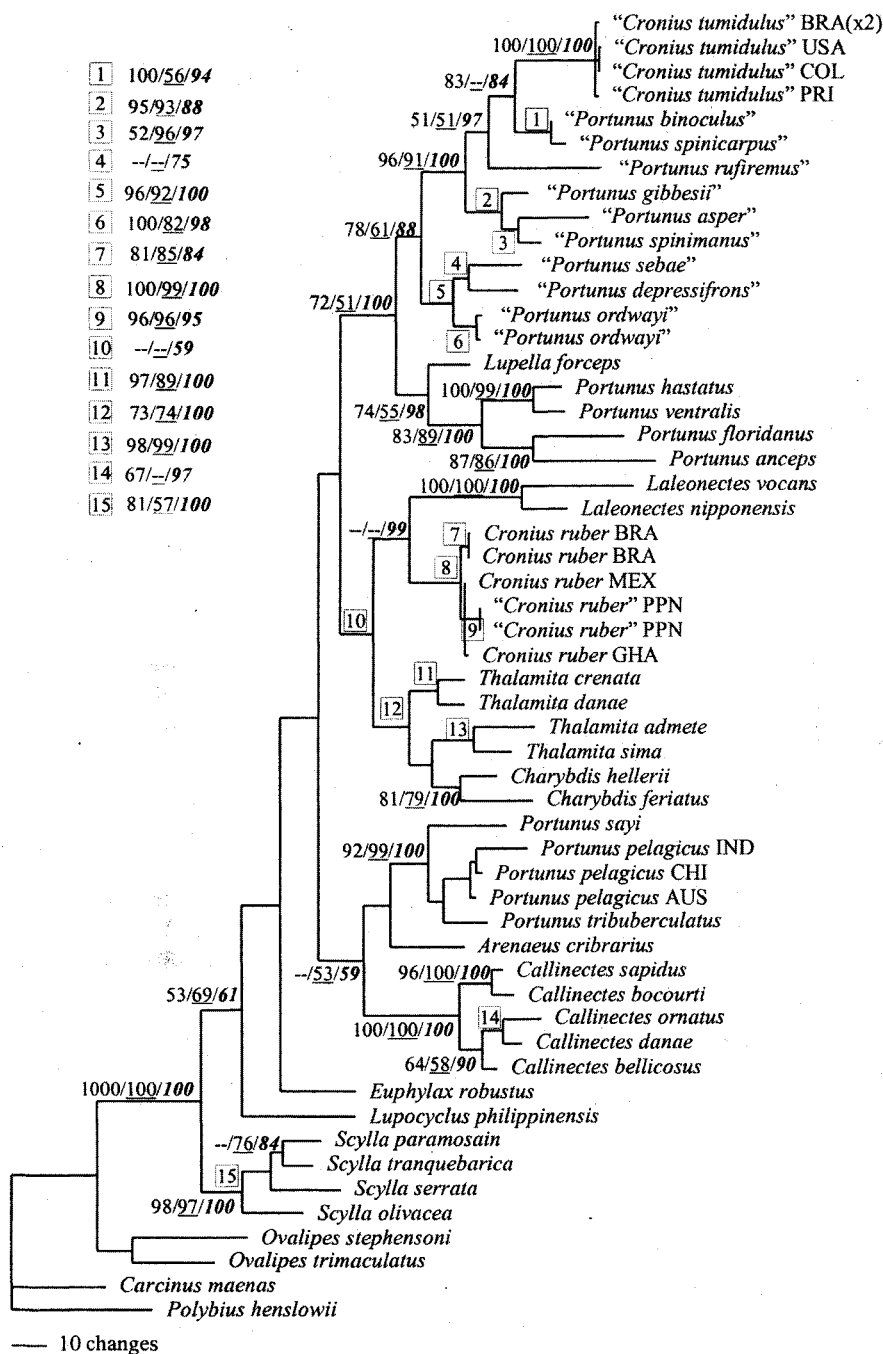


Figure 1. Evolutionary relationships of selected species of Portunidae based on a fragment of the 16S rDNA obtained with BAY analysis. Numbers on nodes are support values for that clade, NJ, MP, and BAY, respectively. Three-letter abbreviations are shown for species for which we obtained sequences from multiple populations (see Table 1 for details). BRA = Brazil, USA = United States of America, COL = Colombia, PRI = Puerto Rico, MEX = Mexico, PPN = Pacific Panama, GHA = Ghana, IND = India, CHI = China, AUS = Australia. Quote marks (" ") are used to show commonly used present names that are proposed for revision in this paper; (x2) indicates two identical sequences from the same locality. The name *C. edwardsii* is resurrected for specimens from the eastern Pacific. Even so, the genetic differences between this species and Atlantic populations are clearly less marked than in some trans-Panamic sister taxa of the genera *Alpheus* Fabricius, 1798 (see Knowlton et al. 1993), *Callinectes* (see Robles et al. 2007), and *Pachygrapsus* Randall, 1839 (see Schubart et al. 2005).

morphologically based classification of *Cronius*. Our analysis places *C. tumidulus* in a different clade from that of *C. ruber*, thus suggesting polyphyly of the genus. *Cronius tumidulus* appears derived from American representatives of the genus *Portunus sensu lato* with which it is clustered with high confidence values (78/61/88). On the other hand, all populations putatively assignable to *C. ruber* are found in a second clade, along with two species of *Laeonectes* and representatives of the Thalamitinae. However, the relationship between populations presently assigned to *C. ruber* and these other genera remains poorly resolved, as basal nodes are weakly supported. The genus *Portunus* is shown to be polyphyletic, with one clade encompassing two groups of species, among which are found all of the included American representatives except for *P. sayi*. Yet another clade contains *P. sayi* and the Indo-West Pacific species, which include *P. pelagicus*, type species of that genus.

Positional differences among putative populations of *Cronius ruber* were very limited. Even so, genetic divergences between Atlantic and Pacific populations are more pronounced (Gulf of Mexico vs. Pacific, 4 transitions [ts] and 1 transversion [tv]; Brazil vs. Pacific, 7 ts and 1 tv; Ghana vs. Pacific, 5 ts and 1 tv) than are divergences between Atlantic populations (Ghana vs. Gulf of Mexico, 1 tv; Ghana vs. Brazil, 4 ts).

4 DISCUSSION

4.1 Paraphyly of *Cronius* and related taxonomic revisions

Paraphyly of *Portunus* was reported previously by Mantelatto et al. (2007) and is corroborated here with treatment of additional taxa. According to our present molecular phylogeny, the genus *Cronius*, as currently defined, is also paraphyletic. We propose a new taxonomy, with restriction of the group defined as the genus *Portunus* and re-elevation of the subgenus *Achelous* De Haan, 1833, to full generic rank. Within *Achelous* (for the present) we include nine American species formerly assigned to the genus *Portunus* and *Cronius tumidulus* (see Table 1). The genus *Achelous* thus contains *A. asper* (A. Milne-Edwards, 1861), *A. binoculus* (Holthuis, 1969), *A. depressifrons* (Stimpson, 1859), *A. gibbesii* (Stimpson, 1859), *A. ordwayi* Stimpson, 1860, *A. rufiremus* (Holthuis, 1959), *A. sebae* (H. Milne Edwards, 1834), *A. spinicarpus* Stimpson, 1871, *A. spinimanus* (Latreille, 1819), and *A. tumidulus* Stimpson, 1871.

On the basis of our molecular genetic analyses of western Atlantic, eastern Atlantic, and eastern Pacific populations presently assigned to *Cronius ruber*, we for now continue to synonymize *C. millerii* with *C. ruber*. The small genetic differences in 16S mtDNA sequences, especially with our small sample size, are not deemed adequate for distinction of the African *C. millerii* as a separate species at this point, and its populations are thus treated under *C. ruber* provisionally. Similarly, Brazilian and Gulf of Mexico populations of *C. ruber* were not deemed to be adequately distinguished to justify separation, though analyses of additional samples and additional genes may warrant reconsideration of this issue in the future. Slightly more substantial genetic differences were found between populations of *C. ruber* from the Pacific American coastline and all of the populations in Atlantic waters. This divergence likely reflects historical separation of Atlantic and Pacific tropical waters by closure of the Panama Isthmus, as has been invoked to explain separations of other marine decapod species pairs or sister taxa (Knowlton & Weight 1998).

As long as genetic homogeneity along both coastlines of the Atlantic remains unknown, it appears premature to recognize separate species for populations of *Cronius ruber* in the eastern and western Atlantic, and we elect to follow morphologically based conclusions (color pattern and ornamentation of the chelae) of Manning & Holthuis (1981). *Cronius ruber* thus has an amphi-Atlantic distribution and a closely related trans-Isthmian sister species. A similar distribution can be found for many other littoral decapod crustaceans, and questions remain whether such largely separated allopatric populations really belong to the same species. *Cronius tumidulus* shows clear genetic separation from *C. ruber* and clearly warrants treatment in a different genus. On the basis of its apomorphic morphological characters, one might assume it deserves treatment in its own genus.

However, its close genetic similarity to nine American representatives of the genus *Portunus*, a morphologically diverse group which is also in need of reclassification (see Mantelatto et al. 2007: fig. 1, clade C, plus *P. asper* from the eastern Pacific in the present work), prompts us to consolidate the taxonomy of this entirely American group by placing them together in one monophyletic genus. By elevating the available subgeneric name *Achelous* for this group, with the American *Portunus spinimanus* as type species of the genus, we alleviate the paraphyly of *Cronius* and partly address the polyphyly of *Portunus*. As treated here, the genus *Achelous* currently encompasses ten species listed above, but with high probability it will eventually include more eastern Pacific forms as studies progress (Mantelatto et al. in preparation). While *P. sayi* is positioned with strong support in a common clade with *P. pelagicus* (type species of the genus) and will thus remain within the genus *Portunus*, the taxonomic position and reclassification of other species of *Portunus* from the western Atlantic [*Portunus anceps* (de Saussure, 1858), *P. ventralis* (A. Milne-Edwards, 1879), and *P. floridanus* Rathbun, 1930] and Mediterranean [*Portunus hastatus* (Linnaeus, 1767)] must await further studies of additional American and western African representatives.

The western Atlantic *C. tumidulus* was originally described by Stimpson (1871) as *Achelous tumidulus*, even though he had also previously erected the genus *Cronius* for *C. ruber* in 1860. We can thus interpret that at least Stimpson did not see a close relationship between the two species. Later, *A. tumidulus* was reclassified under the genus *Neptunus*, as *N. tumidulus* (by A. Milne-Edwards 1879), as *Charybdella tumidula* (by Rathbun 1901), and finally within *Cronius* as *C. tumidulus* (by Rathbun 1920). Only after the present study does it again become a species of the genus to which it had been originally assigned.

Our molecular analysis agrees with recent results obtained from larval morphology (Fransozo et al. 2002). Important differences were noted between the larval morphological characters of *C. ruber* and *C. tumidulus*, which led those authors to cluster zoeae of Portuninae into two subgroups (see also Stuck & Truesdale 1988). Zoeae with relatively long antennal exopods were found typical of *C. tumidulus*, *Portunus gibbesii*, *P. spinicarpus*, and *Scylla serrata*, while those with short antennal exopods were found to represent *Cronius ruber*, *Arenaeus cribrarius*, *Callinectes danae*, *C. sapidus*, and *Charybdis hellerii*. With the exception of *Scylla serrata*, which holds a somewhat intermediate position in terms of larval morphology (see Fransozo et al. 2002: table 1) and a basal position in our molecular phylogeny, the zoeal subgroups correspond perfectly with those grouped by 16S mtDNA; only members of the newly defined *Achelous* have an antennal exopod length equal to or exceeding 1/3 the protopod length.

Rathbun (1930: 34–35) defined morphology of the subgenus *Achelous* in her keys as “Carapace narrow; antero-lateral margin the arc of a circle with short radius, whose center is near the center of the cardiac region,” and for the subgenus *Portunus* as “Carapace wide; antero-lateral margin the arc of a circle with long radius, whose center is near the posterior margin of the carapace.” She indicated *Cronius tumidulus* has a narrower carapace than *C. ruber*, which fits the description of *Achelous*. On the other hand, the defining characters of *Cronius* according to Rathbun (1930: 14) are “Movable portion of antenna excluded from orbit by a prolongation of its basal article. Antero-lateral teeth alternately large and small.” Morphological studies of the representatives of *C. tumidulus* at our disposal did not reveal a clear exclusion of the movable portion of the antenna from the orbit (as opposed to the case in *Cronius*). The presence of alternately large and small anterolateral teeth, on the other hand, is not a character that excludes membership in the subgenera *Portunus* and *Achelous* as defined by Rathbun (1930). We therefore find no morphological contradictions for inclusion of *Cronius tumidulus* within *Achelous*.

The name *Portunus* was originally published by Weber (1795), used by Fabricius (1798), and included practically all the members of the Portunidae known at the time. The history of generic names for species assigned to the genus “*Portunus*” reflects a confused nomenclature, as was previously noted in an extensive revision and synonymy by Palmer (1927). Stephenson & Campbell (1959) built upon this earlier discussion and also gave arguments for and against the use of

subgeneric definitions within this genus. *Achelous* (type species *Portunus spinimanus* Latreille, 1819) has previously been used as one of five valid subgenera within the genus *Portunus*, the others being *Lupocycloporus* Alcock, 1899 [type species *Achelous whitei* A. Milne-Edwards, 1861 = *Portunus gracilimanus* (Stimpson, 1858)], *Monomia* Gistel, 1848 [replacement name for *Amphitrite* De Haan, 1833; type species *Cancer gladiator* Fabricius, 1793], *Portunus* [type species *Cancer pelagicus* Linnaeus, 1758], and *Xiphonectes* A. Milne-Edwards, 1873 [type species *Amphitrite vigilans* Dana, 1852 = *Portunus longispinosus* (Dana, 1852)]. Stephenson & Campbell (1959) noted difficulties in placing four species of *Portunus* in any of the existing subgenera and discussed unresolved relationships with the genus *Callinectes*. They concluded (p. 88): "The real difficulties which arise over the four species above suggest that it is preferable at this stage to avoid the use of subgeneric categories while dealing with the Indo-West Pacific fauna." This suggestion has been followed from then on, not only for the Indo-West Pacific fauna, but also for the genus *Portunus* as a whole (e.g., Crosnier 1962; Türkay 1971; Stephenson 1972; Manning & Holthuis 1981; Williams 1984; Mantelatto et al. 2007). After almost fifty years, we break with this tradition by resurrecting one of the subgenera and elevating it to full generic status, similar to what Barnard (1950) did when using *Achelous*, *Hellenus* (= *Xiphonectes*), *Lupa* (= *Portunus*), and *Monomia* as full genera, into which he classified the South African swimming crabs. We are aware that this is but a first step that does not solve taxonomic issues for the entire genus. Future morphological and molecular systematic work must address whether other subgenera warrant elevation or whether other new genera need to be proposed (for example, as done by Manning 1989 for *Sanquerus* and Ng & Takeda 2003 for *Atoportunus*) in order to provide a natural classification based on monophyletic clades.

Six of the ten species we propose to include in the genus *Achelous* formerly belonged to that taxon as a subgenus (Rathbun 1930; Ng et al. 2008). It is noteworthy that three of them originally were described as species of *Achelous*: *A. ordwayi*, *A. spinicarpus*, and *A. tumidulus*. *Portunus vossi* Lemaitre, 1991, recently synonymized with *A. spinicarpus*, and *P. bahamensis* Rathbun, 1930, recently synonymized with *A. depressifrons* (see Mantelatto et al. 2007), would obviously also represent materials and descriptions now to be associated with *Achelous*. However, *A. asper*, *A. gibbesii*, and *A. rufiremus* have been treated recently as members of the subgenus *Portunus* (see Rathbun 1930; Ng et al. 2008), and their apparent morphological distinction from the other species of *Achelous* should be reexamined to confirm our proposition. The definition used by Stimpson (1860: 221) for *Achelous* differs somewhat from the later one by Rathbun (1930). Stimpson noted the genus to be "chiefly characterized by the shape of the merus-joint of the external maxillipeds, which is greatly produced anteriorly beyond the base of the palpus, with its outer margin usually straight, but sometimes little projecting at the antero-exterior angle." Perhaps this character, in addition to gonopod morphology, should be reconsidered in defining American members of *Portunus*, rather than depending upon vaguely defined differences in carapace shape. Stephenson & Campbell (1959) previously stressed the potential importance of gonopod morphology for subdivision of *Portunus* and provided examples of possible characters in gonopod structure that reflected subgeneric classifications among some Australian species of *Portunus*.

This study is an early step in revising taxonomy of the apparently polyphyletic genus *Portunus*. Not all western Atlantic species of *Portunus* dealt with in Mantelatto et al. (2007) have been addressed in this reclassification, which has focused primarily on those taxa potentially grouped with *Cronius tumidulus* and the resurrected type species of *Achelous*, *A. spinimanus* (those of clade C in Mantelatto et al. 2007). Our phylogeny adds evidence that the phylogenetic position of clade B in Mantelatto et al. (*Portunus anceps*, *P. ventralis*, and *P. floridanus*) requires future clarification, especially our adding of *P. hastatus* to this clade and revealing an apparent basal relationship of the entire clade to the genus *Lupella*. Additional taxa are currently being added to the analysis, with a special effort for broadened coverage of eastern Atlantic and Pacific genera.

4.2 Subfamily considerations and future work

The original description of *Cronius* by Stimpson (1860) suggested this new genus to occupy an intermediate position between *Portunus*, a member of the subfamily Portuninae Rafinesque, 1815, and *Charybdis*, a member of the subfamily Thalamitinae Paulson, 1875. Most taxa of the subfamily Thalamitinae are representatives of two genera that we included in our analysis, *Charybdis* De Haan, 1833 (with approximately 50 species), and *Thalamita* Latreille, 1829 (with approximately 90 species); the remaining genera *Gonioinfradens* Leene, 1938 (one species), and *Thalamitoides* A. Milne-Edwards, 1869 (three species), apply to comparatively few representatives (Fig. 1). We included only six species of this putative subfamily and they resolved as a well-supported monophyletic clade, but it is positioned among different genera of the Portuninae rather than being separated from these at a basal node. At low support levels, *Cronius sensu stricto* and *Laeonectes* are positioned as a sister group to representatives of the Thalamitinae, *Charybdis* and *Thalamita*.

It is tempting to conclude that Thalamitinae simply represents a lineage within Portuninae that is characterized by broader fronts. That conclusion would be in agreement with Rathbun (1930), Stephenson & Campbell (1960), Türkay (1971), and Stephenson (1972), in which case the subfamilies would be synonymous and the name Portuninae would have priority. However, support levels for the basal nodes that position Thalamitinae in the present analysis remain too low for us to confidently draw this conclusion. We thus defer further consideration of this issue until we complete additional molecular analyses currently in progress.

Pending analyses include additional taxa of the aforementioned families, as well as an expanded subset of species representing Polybiinae and Carcininae. Topology of our present tree suggests that Polybiinae (represented by *Polybius* and *Ovalipes*) is polyphyletic, as von Sternberg & Cumberland (2001) have already indicated in their cladistic analysis, but again our present support values are low. The subfamily Polybiinae has been regarded as a basal group in the Portunoidea on the basis of morphological characters (Guinot 1978), zoeal evidence (Rice 1981), and molecular analysis (Mantelatto et al. 2007). Its potential monophyly and phylogenetic position within the family can be addressed only with broader representation of portunoid generic diversity in subsequent analyses.

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