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Decapod Crustacean Phylogenetics

edited by

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Evolutionary Origin of the Gall Crabs (Family Cryptochiridae) Based on 16S rDNA Sequence Data

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ABSTRACT

Gall crabs (family Cryptochiridae) are small brachyuran crabs living on or in depressions formed in scleractinian corals. Their adaptation to this unusual habitat has led to specializations, including mucous feeding, small body size, and relatively short appendages. Currently, gall crabs are treated as constituting a distinct superfamily (Cryptochiroidea) that contains the sole family Cryptochiridae. There has never been an attempt to elucidate the relationships of the gall crabs to other brachyurans. The group is therefore an ideal candidate for employing molecular data to deduce phylogenetic relationships. We sequenced a 545-bp fragment of the 16S mitochondrial gene from specimens of a widespread species of cryptochirid (*Hapalacarcinus marsupialis*) from Mexico and French Polynesia and compared these to other crab sequences available in GenBank. Our preliminary analyses confirm the placement of the cryptochirids in the Brachyura subsection Thoracotremata. Our results also indicate that cryptochirids are members of the superfamily Grapsoidea and are probably closely allied with the family Grapsidae. The Grapsoidea as presently defined is considered a paraphyletic assemblage.

1 INTRODUCTION

Crabs of the family Cryptochiridae Paul'son, 1875, are among the most unusual of all groups of decapod crustaceans. From what little we know about their biology and natural history, it appears that young crabs settle on scleractinian corals, and most species somehow induce the coral to grow over and around the crab. For some cryptochirids, the result is merely a protective indentation or crevice within the coral, and there appears to be little modification of the host. Females, and in some cases males, live in open pits or tunnels in the corals, or on the surface of the corals. Some species (notably *Hapalocarcinus marsupialis* and *Pseudohapalocarcinus ransoni*) live within the protective confines of a coral "gall" that completely or partially (in the case of *Pseudohapalocarcinus*) encompasses and protects the crab, where it remains for the remainder of its life (see Kropp 1986, 1988; Abelson et al. 1991; Carricart-Ganivet et al. 2004 for reviews of species-specific life histories). Males, which are far smaller than females, and about which less is generally known, are also sometimes found in pits or depressions on the same coral (e.g., the crab genus *Fungicola*, which inhabits fungiid corals) or are not directly associated with the coral as far as is known. Currently, the family includes 46 extant species (there are no known fossil species) partitioned among 20 genera (Table 1; see also Ng et al. 2008: 212). Cryptochirids are probably found wherever scleractinian coral reefs occur worldwide, although some reef systems have yet to be rigorously sampled for them. There are also species associated with deep-water, ahermatypic corals found far from reefs. Although roughly circumtropical in distribution, the group is most diverse in the Indo-West Pacific. Table 1 is the first compilation

Table 1. Comprehensive list of described genera (in bold) and species of the family Cryptochiridae, with a summary of the coral families and genera that the crabs inhabit, general biogeographic distributions of the crab genera, and depth records. Depth applies to the entire geographic range.

Genus and Species / Known Coral Hosts	General Distribution (of crab)	Primary References
***Cecidocarcinus* Kropp & Manning, 1987**		
Dendrophylliidae: *Dendrophyllia, Enallopsammia*	Atlantic: Valdivia Ridge (southeastern Atlantic, off Namibia); depth 512 m	Kropp & Manning 1987
Cecidocarcinus brychius Kropp & Manning, 1987		
Cecidocarcinus zibrowii Manning, 1991		
***Cryptochirus* Heller, 1861**		
Faviidae: *Cyphastrea, Barabatoia, Favia, Favites, Goniastrea, Leptoria, Montastrea, Platygyra*	Red Sea	Kropp 1990
Oculinidae: *Cyathelia*	Pacific: Vietnam, Japan, Micronesia (Palau, Guam, Pohnpei); depth <1 to 30 m	Wei et al. 2006
Cryptochirus coralliodytes Heller, 1861		
Cryptochirus planus (Takeda & Tamura, 1983)		
Cryptochirus rubrilineatus Fize & Serène, 1957		
***Dacryomaia* Kropp, 1990**		
Siderastreidae: *Psammocora*	Pacific: Vietnam, Japan (Isu Islands, Ogasawara Islands, Ryukyu Islands), Micronesia (Palau, Guam); depth <1 to 8 m	Kropp 1990 Wei et al. 2006
Dacryomaia edmondsoni (Fize & Serène, 1956a)		
Dacryomaia japonica (Takeda & Tamura, 1981b)		
Dacryomaia sp. 1	Pacific: Micronesia (Guam)	Paulay et al. 2003
Dacryomaia sp. 2	Pacific: Micronesia (Guam)	Paulay et al. 2003
***Detocarcinus* Kropp & Manning, 1987**		
Caryophylliidae: *Asterosimilia, Caryophyllia*	Atlantic: off Ghana	Kropp & Manning 1987
Dendrophylliidae: *Dendrophyllia* (questionable)		
Oculinidae: *Schizoculina*		
Rhizangiidae: *Phyllangia*		
Detocarcinus balssi (Monod, 1956)		

Table 1. continued.

Genus and Species Known Coral Hosts	General Distribution (of crab)	Primary References
***Fizesereneia* Takeda & Tamura, 1980b**		
Mussidae: *Acanthastrea, Lobophyllia, Symphyllia*	Pacific: Vietnam, Indonesia, Japan (Izu Islands, Ryukyu Islands), Australia, Micronesia (Palau, Guam, Pohnpei); depth 1 to 15 m	Kropp 1990
Fizesereneia heimi (Fize & Serène, 1956a)		
Fizesereneia ishikawai (Takeda & Tamura, 1980b)		
Fizesereneia latisella Kropp, 1994		
Fizesereneia stimpsoni (Fize & Serène, 1956b)		
Fizesereneia tholia Kropp, 1994		
***Fungicola* Serène, 1966**		
Fungiidae: *Fungia, Podobacia, Sandalolitha*	Pacific: Vietnam, Indonesia, Japan (Ryukyu Islands), Micronesia (Palau, Guam); depth 1 to 15 m	Kropp 1990
Fungicola fagei (Fize & Serène, 1956a)		
Fungicola utinomii (Fize & Serène, 1956a)		
***Hapalocarcinus* Stimpson, 1859**		
Pocilloporidae: *Pocillopora, Seriatopora, Stylophora*	Pacific: Indo-West Pacific to Eastern Pacific (Colombia) Red Sea; depth 1 to 27 m	Kropp 1990 Wei et al. 2006
Hapalocarcinus marsupialis Stimpson, 1859		
***Hiroia* Takeda & Tamura, 1981a**		
Faviidae: *Cyphastrea, Hydnophora* Merulinidae: *Merulina*	Pacific: Vietnam, Japan (Izu Islands, Ryukyu Islands), Micronesia (Palau, Guam); depth 1 to 19 m	Kropp 1990 Wei et al. 2006

Table 1. continued.

Genus and Species **Known Coral Hosts**	**General Distribution (of crab)**	**Primary References**
Hiroia krempfi (Fize & Serène, 1956a)		
***Lithoscaptus* Milne Edwards, 1862**		
Faviidae: *Cyphastrea, Echinopora, Favia, Favites, Hydnophora, Goniastrea, Leptastrea, Platygyra, Plesiastrea*	Pacific: Réunion, Vietnam, Japan (Izu Islands, Kushimoto, Ogasawara Islands, Ryukyu Islands), Micronesia (Palau, Guam, Pohnpei), Palmyra Island, Teraina; depth <1 to 12 m	Kropp 1990 Wei et al. 2006
Merulinidae: *Merulina*		
Lithoscaptus grandis (Takeda & Tamura, 1983)		
Lithoscaptus helleri (Fize & Serène, 1957)		
Lithoscaptus nami (Fize & Serène, 1957)		
Lithoscaptus (?) *pacificus* (Edmondson, 1933)[1]		
Lithoscaptus paradoxus Milne Edwards, 1862		
Lithoscaptus pardalotus Kropp, 1995		
Lithoscaptus prionotus Kropp, 1994		
Lithoscaptus tri (Fize & Serène, 1956b)		
***Luciades* Kropp & Manning, 1996**		
Pavonidae: *Leptoseris*	Pacific: Micronesia (Guam); depth 128 to 137 m	Kropp & Manning 1996
Luciades agana Kropp & Manning, 1996		
***Neotroglocarcinus* Takeda & Tamura, 1980a**		
Dendrophyllidae: *Turbinaria*	Pacific: Vietnam, Japan (Izu Islands, Ryukyu Islands), Micronesia (Palau, Guam, Pohnpei), Enewetak, Hong Kong; depth <1 to 13 m	Kropp 1990 Wei et al. 2006
Neotroglocarcinus hongkongensis (Shen, 1936)		
Neotroglocarcinus dawydoffi (Fize & Serène, 1956a)		

Table 1. continued.

Genus and Species Known Coral Hosts	General Distribution (of crab)	Primary References
***Opecarcinus* Kropp & Manning, 1987**		
Agariciidae: *Agaricia, Gardineroseris, Leptoseris, Pavona* Siderasteriidae: *Coscinaraea, Siderastrea*	Pacific: Vietnam, Japan, to west coast of Mexico Indian Ocean: Christmas Island Atlantic Ocean: Ascension Island and western Atlantic (Caribbean, Gulf of Mexico south to Brazil); depth <1 to 82 m	Kropp & Manning 1987 Kropp 1990 Wei et al. 2006
Opecarcinus aurantius Kropp, 1989		
Opecarcinus crescentus (Edmondson, 1925)		
Opecarcinus granulatus (Shen, 1936)		
Opecarcinus hypostegus (Shaw & Hopkins, 1977)		
Opecarcinus lobifrons Kropp, 1989		
Opecarcinus peliops Kropp, 1989		
Opecarcinus pholeter Kropp, 1989		
Opecarcinus sierra Kropp, 1989		
***Pelycomaia* Kropp, 1990**		
Faviidae: *Cyphastrea, Leptastrea*	Pacific: Vietnam, Micronesia (Guam), Hawaii; depth < 2 m	Kropp 1990
Pelycomaia minuta (Edmondson, 1933)		
***Pseudocryptochirus* Hiro, 1938**		
Dendrophylliidae: *Turbinaria*	Pacific: Vietnam, Indonesia, Japan (Isu Islands), Micronesia (Palau, Guam, Pohnpei); depth 1 to 6 m	Kropp 1990 Wei et al. 2006
Pseudocryptochirus viridis Hiro, 1938		

Table 1. continued.

Genus and Species Known Coral Hosts	General Distribution (of crab)	Primary References
***Pseudohapalocarcinus* Fize & Serène, 1956a**		
Agariciidae: *Pavona*	Pacific: Vietnam, Japan (Ryukyu Islands), Micronesia (Palau, Guam, Pohnpei); depth <1 to 21 m	Kropp 1990
Pseudohapalocarcinus ransoni Fize & Serène, 1956a		
***Sphenomaia* Kropp, 1990**		
Faviidae: *Favites, Hydnophora, Platygyra*	Central Pacific (Teraina); depth not recorded	Kropp 1990
Sphenomaia pyriforma (Edmondson, 1933)		
***Troglocarcinus* Verrill, 1908**		
Astrocoeniidae: *Stephanocoenia* Caryophylliidae: *Polychathu* Faviidae: *Diploria, Manicina* Meandrinidae: *Dichocoenia* Mussidae: *Isophyllia, Mussa, Mussimilia, Mycetophyllia, Scolymia* Oculinidae: *Oculina* Siderastreidae: *Siderastrea*	Atlantic: Bermuda, Florida, Caribbean south to Brazil, Ascension Island, eastern Atlantic; depth <1 to 75 m	Kropp & Manning 1987
Troglocarcinus corallicola (Fize & Serène, 1956a)		Carricart-Ganivet et al. 2004
***Utinomiella* Kropp & Takeda, 1988**		
Pocilloporidae: *Pocillopora, Stylophora*	Pacific: Japan (Ryukyu Islands), Micronesia (Palau, Guam, Pohnpei), Hawaii Indian Ocean: Andaman Islands; depth 1 to 29 m	Kropp 1990 Wei et al. 2006
Utinomiella dimorpha (Henderson, 1906)		

Table 1. continued.

Genus and Species **Known Coral Hosts**	**General Distribution (of crab)**	**Primary References**
***Xynomaia* Kropp, 1990**		
Faviidae: *Favia, Goniastrea, Montastrea, Oulophllia, Platygyra* Merulinidae: *Merulina* Pectiniidae: *Pectinia*	Pacific: Vietnam, Sumatra, Japan (Izu Islands, Kushimoto), Micronesia (Palau, Guam); depth 1 to 15 m	Kropp 1990
Xynomaia boissoni (Fize & Serène, 1956a)		
Xynomaia sheni (Fize & Serène, 1956b)		
Xynomaia verrilli (Fize & Serène, 1957)		
***Zibrovia* Kropp & Manning, 1996**		
Phyllangiidae: *Phyllangia*	Pacific: Philippines Indian Ocean: Madagascar; depth 81 to 100 m	Kropp & Manning 1996
Zibrovia galea Kropp & Manning, 1996		

[1] The question mark after the genus name in *Lithoscaptus pacificus* refers to the fact that, because of the poor condition of the type of *Cryptochirus pacificus* Edmondson, Kropp (1990) placed the species in the genus *Lithoscaptus* only tentatively.

that includes all genera and species of the family, the host scleractinian coral genus from which they have been reported, and the general distribution patterns of each cryptochirid genus.

Presumably as an adaptation to their environment (their close association with corals), the cryptochirids have evolved a small, squat, and distinctive body that, although perhaps superficially similar to crabs of the family Pinnotheridae in some species, is unlike that of other crab families, even those that also live as obligate commensals of corals (e.g., trapeziids and domeciids). Based on their morphology, in the most current (and indeed in all other) classifications, the gall crabs are placed in their own family (Cryptochiridae) and superfamily (Cryptochiroidea). There is some (unpublished) information indicating that the family is probably monophyletic (Kropp 1988), but little beyond that. Even placement of the superfamily within the Eubrachyura (higher crabs) has been historically uncertain. For example, Martin & Davis (2001) placed the gall crabs within the subsection Heterotremata, whereas the most recent treatment of the Brachyura (Ng et al. 2008) places the superfamily Cryptochiroidea in the subsection Thoracotremata. It would seem, therefore, that the question of the origin and evolutionary relationships of the cryptochirid crabs is a question perfectly suited to investigation with molecular systematic techniques. We address for the first time the evolutionary relationships of gall crabs to other brachyuran families using molecular sequence data. This study must be considered preliminary in that only two populations of a single species (the widespread *Hapalocarcinus marsupialis* Stimpson, 1859) were included, but the results seem sufficiently robust to suggest affinities of the gall crabs at the superfamily and possibly family level.

2 MATERIALS AND METHODS

We sequenced a ~545-bp fragment of the 16S mitochondrial gene from Mexican and French Polynesian specimens of the cryptochirid *Hapalocarcinus marsupialis* Stimpson, 1895. The Mexican material was extracted from crabs removed from corals that had been in the collections of the Natural History Museum of Los Angeles County. The Polynesian material was collected in 2001 and was preserved in ethanol. Locality and collection details as well as GenBank numbers are included in Table 2. Muscle tissue was taken from the fifth pereopod and was extracted with a QIAGEN DNeasy Kit (Qiagen, Valencia, CA). The manufacturer's protocol was followed for extraction, and tissue was macerated in a PCR tube with a pestle and then incubated in a 55°C incubator overnight on a shaking table set to medium speed. Polymerase chain reaction (PCR, Sakai et al. 1988) was carried out with standard PCR conditions (2.5 μl of 10x PCR buffer, 1.5 μl of 50 mM $MgCl_2$, 4 μl of 10 mM dNTPs, 2.5 μl each of two 10 pmol primers, 0.15 Platinum *Taq* (5 units/μl), 9.6 μl double distilled water, and 1 μl template) and thermal cycling as follows: an initial denaturation at 96°C for 3 minutes followed by 40 cycles of 95°C for 1 minute, 46°C for 1 minute, and 72°C for 10 minutes. 16SrDNA was amplified in both directions with universal 16Sar and 16Sbr primers (Palumbi et al. 1991). PCR products were visualized by agarose (1.2%) gel electrophorsis with Sybr Gold (Invitrogen, Carlsbad, CA), PCR product was purified with Sephadex (Sigma Chemical, St. Louis, MO) on millipore multiscreen filter plates, and DNA was cycle sequenced with ABI Big-dye ready-reaction kit and following the standard cycle sequencing protocol with one quarter of the suggested reaction volume.

Sequences were edited and assembled in Sequencher (Gene Codes Corporation); 16S rDNA was aligned using MAFFT (Multiple Alignment Program for amino acid or nucleotide sequences, Katoh et al. 2002; Katoh et al. 2005) and manually adjusted where mismatches were made. All three LINS, EINS, and GINS alignment protocols were reviewed. Phylogenetic trees were estimated with maximum likelihood (GARLI, Genetic Algorithm for Rapid Likelihood Inference, Zwickl 2006). GARLI phylogenetic searches on aligned nucleotide datasets begin with an assumed model of nucleotide substitutions (GTR), with gamma distributed rate heterogeneity and an estimated proportion of invariable sites. The implementation of this model is exactly equivalent to that in PAUP*, making the log likelihood (lnL) scores obtained directly comparable. All model parameters were estimated, including the equilibrium base frequencies. The gamma model of rate heterogeneity

Table 2. Cryptochirids sequenced and GenBank sequences used in analyses.

Subsection Superfamily	Family	Genus/species	GenBank No.
Cryptochiroidea			
	Cryptochiridae	*Hapalocarcinus marsupialis*	EU743929
Mexico, Baja California Sur, Palmas Bay, Rancho Buena Vista, *Pocillopora* with barnacles, 4.57 m. Original fixative unknown, specimen in 70% ethanol. 15 Sep. 1962. AHF, 1963-13, lot 13, cat. no. 530, JM-2005-003. Coll. Edmond Hobsen. RW05.301.1154.			
			EU743930
Pacific, Society Islands, French Polynesia, Moorea, 6 km south of airport, site 9, ~17.533° S ~149.783° W, *Pocillopora* with barnacles, snorkel to motu, very close to outer reef, original fixative rum 50% ethanol, subsequently transfered to 95% ethanol. 25 Jul. 2001. JM-2005-004, ST01.055. Coll. Sandy Trautwein. RW05.302.1155.			
Heterotremata			
Potamoidea	Gecarcinucidae	*Sartoriana spinigera*	AM234649
	Potamidae	*Geothelphusa pingtung*	AB266168
Thoracotremata			
Grapsoidea	Gecarcinidae	*Cardisoma carnifex*	AM180687
		Gecarcinus lateralis	AJ130804
		Gecarcoidea lalandii	AM180684
	Glyptograpsidae	*Glyptograpsus impressus*	AJ250646
		Platychirograpsus spectabilis	AJ250645
	Grapsidae	*Geograpsus lividus*	AJ250651
		Goniopsis cruentata	AJ250652
		Grapsus grapsus	AJ250650
		Leptograpsus variegatus	AJ250654
		Metopograpsus latifrons	AJ784028
		Metopograpsus quadridentatus	DQ062732
		Metopograpsus thukuhar	AJ784027
		Pachygrapsus crassipes	AB197814
		Pachygrapsus marmoratus	DQ079728
		Pachygrapsus minutus	AB057808
		Pachygrapsus transversus	AJ250641
		Planes minutus	AJ250653
	Plagusiidae	*Euchirograpsus americanus*	AJ250648
		Percnon gibbesi	AJ130803
		Plagusia squamosa	AJ311796
	Sesarmidae	*Armases elegans*	AJ784011
		Sarmatium striaticarpus	AM180680
		Sesarma meridies	AJ621819
		Sesarma windsor	AJ621824
		Sesarmoides longipes	AJ784026
	Varunidae	*Austrohelice crassa*	AJ308416
		Brachynotus atlanticus	AJ278831
		Cyrtograpsus affinis	AJ130801
		Eriocheir sinensis	AJ250642
		Gaetice americanus	AJ250643
		Helograpsus haswellianus	AJ308417
		Hemigrapsus oregonensis	AJ250644

Table 2. continued.

Subsection Superfamily	Family	Genus/species	GenBank No.
		Hemigrapsus sanguineus	AJ493053
		Paragrapsus laevis	AJ308418
		Varuna litterata	AJ308419
Ocypodoidea	Camptandriidae	*Baruna trigranulum*	AB002129
		Paracleistostoma depressum	AB002128
	Mictyridae	*Mictyris brevidactylus*	AB002133
	Ocypodidae	*Dotilla wichmanni*	AB002126
		Ilyoplax deschampsi	AB002117
		Scopimera globosa	AB002125
		Tmethypocoelis ceratophora	AB002127
	Palicidae	*Crossotonotus spinipes*	AJ130807
		Palicus caronii	AM180692
Pinnotheroidea	Pinnotheridae	*Austinixa hardyi*	AF503185
		Austinixa patagoniensis	AF503186
		Pinnotheres pisum	AM180694

assumes four rate categories. GARLI uses a genetic algorithm approach to simultaneously find the topology, branch lengths, and model parameters that maximize the lnL (Zwickl 2006).

The phylogeny was also estimated with Mr. Bayes 3.0b4 (Ronquist & Hulsenbeck 2003) using Bayesian inferences coupled with Markov chain Monte Carlo techniques. Four Markov–Monte Carlo chains were run for ten million generations, and a sample tree was saved every 1000 generations. Trees chosen from the first one million generations were discarded as "burn in." Trees that were chosen once likelihood scores converged on a stable value were used to construct a 50% majority rule consensus tree in PAUP*.

A ~1860-bp double-stranded fragment of 18SrDNA was also sequenced but not used due to a lack of sequence variation (GenBank numbers EU743931 and EU743932). Taxon selection for the analyses was repeatedly refined, as it was determined that Cryptochiridae are members of Thoracotremata and the Grapsoidea and are nested within the Grapsidae. This realization changed our approach from focusing on 18S rDNA to the more appropriate 16S rDNA for this analysis. Taxa selected for the 16S dataset included broad, but not exhaustive, sampling of Varunidae, Grapsidae, Plagusiidae, Sesarmidae, Camptandridae, Gecarcinidae, Pinnotheridae, and Mictridae, with the goal of associating the Cryptochiridae with its closest relatives.

3 RESULTS

Analyses of our cryptochirids from Mexico and Polynesia revealed that despite their geographic separation, both samples were the same species, the widespread and relatively common *Hapalocarcinus marsupialis* Stimpson, 1859. In all of our analyses, the cryptochirids are nested within a group of crabs considered by most workers to constitute the Thoracotremata. More specifically, the genus *Hapalocarcinus* falls within a clade that includes the familiar grapsid genera *Grapsus*, *Geograpsus*, *Goniopsis*, *Leptograpsus*, *Planes*, and *Pachygrapsus* (Fig. 1). Branch lengths for the two *Haplocarcinus* sequences are long, as is the branch length of the *Mictyris* sequence (not shown). Interestingly, however, *Hapalocarcinus* was not close to some of the grapsoids that are common reef inhabitants, such as the genera *Percnon* and *Plagusia*, both of which were at one time considered members of the family Plagusiidae (but see below). Beyond our observations on the gall crabs (based on this single species), our results also indicate that the genus *Pachygrapsus* is not monophyletic, with *P. marmoratus* not clustering with the other four *Pachygrapsus* species.

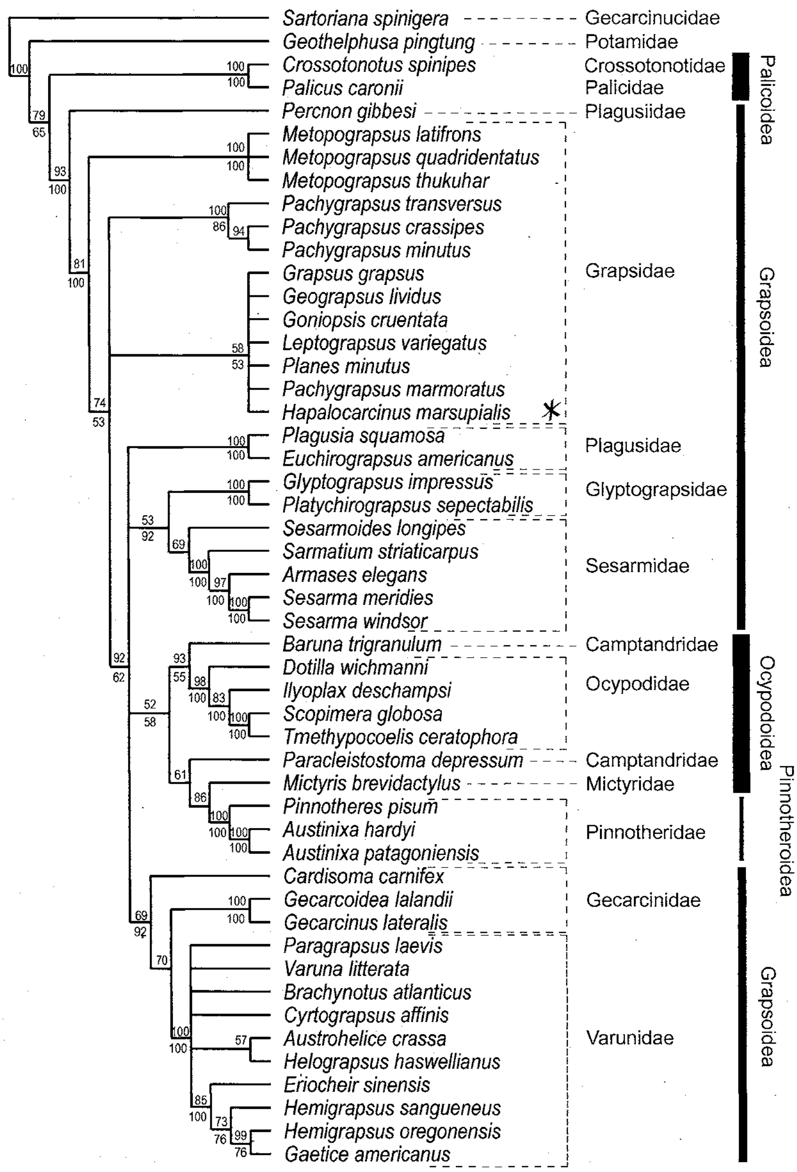

Figure 1. Phylogenetic placement of the Cryptochiridae, represented by the genus *Hapalocarcinus* (*), and relationships of Ocypodoidea, Grapsoidea, Pinnotheroidea, and Palicoidea based on 16S mtDNA sequences of 51 taxa, 589 characters, nucleotide frequences: f(A) = 0.24387, f(C) = 0.24433, f(G) = 0.27220, f(T) = 0.23960. This tree is rooted in Gecarcinidae and Potamidae. Topology derived from Bayesian inference 50% majority rule consensus of 18,000 trees. Significance values are posterior probabilities >50% above the branches. GARLI maximum likelihood ln score = -8935.92, 50% majority rule consensus of 74 trees; bootstrap values are below the branches.

Maximum likelihood and Bayesian analyses converged on the same topology. All of our analyses recognize *Glyptograpsus* and *Platychirograpsus* as sister taxa, confirming their placement in the family Glyptograpsidae. The species of *Pinnotheres* and *Austinixa* selected for this analysis constitute a monophyletic clade (the Pinnotheridae). The Varunidae (*Austrohelice*, *Brachynotus*, *Cyrtograpsus*, *Eriocheir*, *Gaetice*, *Helograpsus*, *Hemigrapsus*, *Paragrapsus*, and *Varuna*) is a well-supported monophyletic clade. Gecarcinidae are basal to the Varunidae (posterior probability 69% and bootstrap support 92%). As alluded to above, the plagusiid genera *Plagusia* and *Euchirograpsus* are sister taxa, but they are not at all closely related to the genus *Percnon*, previously included in the Plagusiidae.

At the superfamily level, Pinnotheroidea appears monophyletic, although only three taxa were used in our analysis. The Palicoidea appears as monophyletic and basal to the "grapsoids" in our phylogeny. In our analysis, the superfamilies Ocypodoidea and Grapsoidea are not monophyletic clades.

4 DISCUSSION

As noted earlier, in all of our analyses, which must be considered preliminary because of the single species used to represent the gall crabs, the cryptochirids are nested within a group of crabs considered by most workers to constitute the Thoracotremata. This group is defined primarily by having the location of the opening of the vas deferens through the sternum rather than through the coxa of the fifth pereopod (Ng et al. 2008: 8). This placement agrees with the most recent compilation and classification of crabs by Ng et al. 2008 and not with the classification suggested by Martin & Davis (2001), in which the cryptochirids were treated as members of the more diverse Heterotremata. The Ng et al. (2008) classification treats the Thoracotremata as being composed of 17 extant families distributed among four superfamilies: Cryptochiroidea, Grapsoidea, Ocypodoidea, and Pinnotheroidea.

Within the Thoracotremata, our best tree places the gall crab genus *Hapalocarcinus* within a clade that includes the familiar grapsid genera *Grapsus*, *Geograpsus*, *Goniopsis*, *Leptograpsus*, *Planes*, and *Pachygrapsus*. Since only a single species was sampled in the family, the long branch length of *Haplocarcinus* precludes more accurate placement within the grapsids in this analysis. The association of *Haplocarcinus* with grapsid genera is a somewhat surprising result, in part because there are other groups of crabs that are closely associated with reefs (e.g., trapeziids, domeciids, and some other coral-associated taxa). Also surprising to us was that, even among grapsoids, there are genera more typically associated with reef-dwelling than those with which *Hapalocarcinus* clusters, such as *Percnon* and *Plagusia*; these were not close to the gall crabs in our results. The transition from a coral-obligate commensal group of crabs (such as the trapeziids, tetraliids, or domeciids) to a more heavily coral-dependent group such as the gall crabs would have been, in some ways, easier to understand. However, no such coral-obligates are seen among the crabs that appear closest to *Hapalocarcinus* in our analysis. We should also point out that adaptation to a coral-associated lifestyle does not always result in similar modifications, even among decapods (e.g., consider the morphological differences between trapeziids and domeciids such as *Maldivia*, or between the shrimp genera *Paratypton* and *Alpheus*) despite similar lifestyles and diets.

Some traditional groupings, such as the families Varunidae, Pinnotheridae, Ocypodidae, Sesarmidae, and Glyptograpsidae, are supported in this analysis. However, other traditionally recognized families, such as the Camptandriidae and Plagusiidae, are not supported (see also Schubart et al. 2002; Schubart et al. 2006). Although a case could be made for recognition of the superfamily Pinnotheroidea, and possibly the Ocypodoidea (with the exception of the genera *Paracleistostoma* and *Mictyris*), there is no support for the superfamilies Cryptochiroidea, Grapsoidea, and Ocypodoidea as previously defined (Fig. 1). This perhaps is not surprising in light of the rather weak and likely convergent morphological characters that have been used to define these superfamilies in the past (such as the "rectangular" carapace shape of the grapsoids and the long eyestalks of many ocypodoids).

The pinnotherids, all of which are highly modified (most having extremely short and wide bodies) for a commensal existence, appear to be monophyletic and are not closely related to cryptochirids despite an apparently superficial resemblance (see Introduction), although this result is based on only three representatives of that family. The former family Palicidae (*Crossotonotus* + *Palicus*) (now treated as two families, Crossotonotidae and Palicidae, within a superfamily Palicoidea; Ng et al. 2008) appears basal to the other (non-outgroup) crabs in our study. Palicids are morphologically very unusual in that they have greatly reduced fifth pereopods (see Castro 2000).

Our results are in general agreement with the findings of Schubart et al. (2002, 2006) in their studies of the Glyptograpsidae and of the relationships within the Grapsoidea, respectively. As in the conclusion of Schubart et al. (2006), our results cast doubt on the usefulness of the superfamily categories Grapsoidea and Ocypodoidea, and confirm that *Percnon* is not allied to *Plagusia* and *Euchirograpsus*, such that the family Plagusiidae cannot be recognized as monophyletic.

For the gall crabs, the superfamily status of the Cryptochiroidea is now difficult to justify, as, based on our admittedly small dataset, the gall crabs appear to be highly modified grapsids. For practical reasons, and until more cryptochirid sequences from a broader family sampling are included in future analyses, we suggest maintaining the family status of the Cryptochiridae but treating it as one of many separate "grapsoid" families. We recommend dropping the superfamily category (Cryptochiroidea), while at the same time recognizing that the Grapsoidea, as previously defined, is itself an artificial assemblage. The rather wide geographical range of the gall crabs, summarized in Table 1, and the fact that, despite the geographical distance between the populations sampled in this study (Mexico and French Polynesia), our sequences came from a single species, also are reasons to suspend making any higher-level classificatory changes, as it is possible that convergence to a coral-dwelling habitat has occurred more than once.

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