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Phylogenetic relationships within the coral crab genus *Carpilius* (Brachyura, Xanthoidea, Carpiliidae) and of the Carpiliidae to other xanthoid crab families based on molecular sequence data

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Abstract

The coral crab genus *Carpilius* currently includes three widely distributed species that inhabit tropical coral reefs and adjacent waters. The relationship of *Carpilius* to other xanthoid crabs is unknown. Previously, carcinologists considered *Carpilius* to be allied with crabs of the family Xanthidae (e.g., *Euryozius*, *Liagore*, and *Liomera*), however, recent workers have considered it to be a monotypic genus within its own family, Carpiliidae. Mitochondrial 12S- and 16S-rDNA gene fragments confirm the monophyly and distinct status of the family Carpiliidae. Within the genus *Carpilius*, the Caribbean species *C. corallinus* is basal to the two Pacific species *C. maculatus* and *C. convexus*. The Pacific species are sister taxa, despite the greater morphological resemblance of *C. corallinus* to the Pacific *C. convexus*. The relationship of the Carpiliidae (*Carpilius*) to other xanthoid crabs is investigated, and results of a preliminary analysis of higher xanthoid relationships did not resolve the relationships of Carpiliidae, "Xanthidae," Menippidae, Trapeziidae, and Ocypodidae to one another. A Menippidae and *Carpilius* relationship could not be rejected, although a *Liomera*, *Liagore*, and *Carpilius* relationship was.
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1. Introduction

1.1. Review of taxonomy and distribution

The brachyuran crab genus *Carpilius*, originally erected by Leach (in Desmarest, 1823), currently consists of three species. All are large and brightly colored crabs associated with tropical coral reefs and adjacent habitats. Probably because of their size and coloration, all three species were already recognized by the 18th century.

Carpilius maculatus, the most distinctive member of the genus because of its large, red, nearly circular spots on the carapace, was recognized by Rumphius (1705) and originally described (as *Cancer maculatus*) by Linnaeus (1758). A large crab reaching 152 mm in carapace width (Tinker, 1965), this species has been reported from the Hawaiian Islands, the far western Pacific where it is widespread, the Indian Ocean, and the Red Sea.

Carpilius convexus was originally described (as *Cancer convexus*) by Forskål (1775). Like *C. maculatus*, this species has been reported from Hawaii, the Indo-Pacific, the Indian Ocean south to Mozambique and South Africa, and the Red Sea (e.g., Galil and Vannini, 1990). Although *C. convexus* differs from *C. maculatus* in its coloration and smaller size, at least one earlier worker (Paul'son, 1875, English translation published in 1961) questioned whether the species were distinct, as he felt that the variability in color had "absolutely no importance in the determination of species" and that the slight

morphological differences were "of too little importance for the creation of a new species"; he (Paul'son, 1961, p. 31) therefore considered *C. convexus* a variety of *C. maculatus*. Subsequent authors, however, have continued to treat *C. maculatus* and *C. convexus* as distinct species.

Carpilius corallinus, originally described (as *Cancer corallinus*) by Herbst (1783), is similar in size to *C. maculatus* (up to 154 mm carapace width) and is widespread throughout the Caribbean, the Gulf of Mexico, and the tropical western Atlantic, with records from Bermuda (Chace et al., 1986), the Gulf of Mexico off Texas (Pequegnat and Ray, 1974), the Bahamas (Rathbun, 1930), and off Sao Paulo, Brazil (Melo et al., 1998).

1.2. Natural history

Despite their size, striking coloration, and economic potential [species of *Carpilius* are eaten in some parts of their range (see Guinot, 1967; Rathbun, 1906, 1930)], little is known about their biology. Pequegnat and Ray (1974) recorded the only known observations of mating behavior and noted that *C. corallinus* feeds on the urchin *Diadema* at night. Based on personal observations (JWM), *C. corallinus* is most commonly observed at night. Similar nocturnal activity is presumed to occur in the Pacific species (Guinot, 1967). Laughlin et al. (1983) briefly described larval development in *C. corallinus*; these authors reported that the species passes through five zoeal stages. All other xanthoid crabs for which larval development has been described have four zoeal stages, with the exception of stone crabs (*Menippe*), which, like *Carpilius*, have five zoeal stages. The megalopa stage of *Carpilius* remains undiscovered.

1.3. Relationship to other brachyurans

Ortmann (1893) originally created the subfamily Carpilinae to contain *Carpilius* as well as the genera *Phymodius*, *Chlorodius*, *Euxanthus*, *Hypocoelus*, and *Carpilodes*. Alcock (1898) subsequently created the "Alliance Carpiloida," which contained his subfamily Xanthinae (Section Hyperolissa) and the genera *Carpilius*, *Carpilodes*, *Liomera*, *Lioxantho*, *Liagore*, and *Lachnopodus*. Guinot (1968) treated three extant genera (*Carpilius*, *Euryozius*, and *Gardineria*) and two fossil genera (*Paleocarpilius* and *Ocalina*) as members of the subfamily Carpilinae Ortmann. Later, Guinot (1978) treated this assemblage as a full family, containing only the extant genus *Carpilius* and the fossil taxa *Paleocarpilius* and *Ocalina*. Crosnier (1984), followed by Poupin (1994) and others (e.g., Ng, 1998), also felt that the distinct nature of *Carpilius* warranted family rank, and he treated the three *Carpilius* species as the monogeneric family Carpiliidae. Affinities with the xanthoid

genus *Menippe* might also seem plausible in light of similarities in larval development (see above) and similarities in pleopod morphology (Guinot, 1978). Most recently, Schweitzer (2000) followed Guinot's (1968) lead in placing three extant genera (*Carpilius*, *Euryozius*, and *Gardineria*) in the Carpiliidae (apparently unaware that *Gardineria* is now considered a junior synonym of *Euryozius*; see Manning and Holthuis, 1981), and added to Guinot's two fossil genera (*Paleocarpilius* and *Ocalina*) the genera *Proxicarpilius*, *Harpactoxanthopsis*, and *Eocarpilius*.

The validity of *Carpilius* species, species relationships, and relationships to other xanthids (or xanthoids) is based on narrative-type scenarios of evolutionary history and remain untested. In this paper, we use mitochondrial 12S- and 16S-rDNA nucleotide sequences to examine the relationships within the genus *Carpilius*, the boundaries of the Carpiliidae, and the relationships of carpiliids to several putative related xanthoids.

2. Materials and methods

2.1. Taxon sampling and tissue extraction

Taxa and localities sampled are listed in Table 1. Twenty frozen or ethanol-preserved (70–95%) specimens of *Carpilius* (10 *C. convexus*, five *C. maculatus*, and five *C. corallinus*) from the Caribbean, coastal Brazil, central and western Pacific, and Red Sea were sequenced. We also sequenced two specimens each of the genera *Liomera* Dana, 1851 and *Liagore* DeHaan, 1835 and one specimen of the giant Tasmanian crab *Pseudocarcinus gigas* (Lamark, 1983). Additional sequences included in the analyses were obtained from GenBank and included all available xanthoids (GenBank numbers for 16S-rDNA sequences: *Dyspanopeus sayi* U75270, *Menippe adina* U20751, *M. mercenaria* U20749, *M. nodifrons* AJ130817, *Panopeus herbstii* AJ130815, *Trapezia cymodoce* AJ130816, and *Xantho poressa* AJ130814). A broad assortment of ocypodids, portunids, and other brachyuran crabs were included for the in- and outgroup analyses. Sequences used in the analyses and their GenBank numbers were as follows:

16S-rDNA sequences: Grapsoidea *Pachygrapsus transversus* AJ250641; Ocypodoidea *Dotilla wichmanni* AB002126, *Ilyoplax dentata* AB002123, *I. deschampsi* AB002117, *I. pingi* AB002119, *I. pusilla* AB002113, *I. tansuiensis* AB002114, *Ocypode stimpsoni* AB002131, *Scopimera bitympana* AB002125, *S. globosa* AB002124; Portunoidea *Callinectes ornatus* U75268, "*C. sapidus*" U75267 (corrected to *C. similis* by Schubart et al., 2001), AJ130813, *C. similis* U75269, *Scylla olivacea* AF109321, *S. paramamosain* AF109319, *S. serrata* AF109318, *S. tranquebarica* AF109320; Astacoidea *Astacus astacus*

Table 1
Specimens examined, GenBank accession numbers for genes sequenced, and sources of material

Family	Genus	Species	Reference No.	16S-rDNA GenBank Accession No.	12S-rDNA GenBank Accession No.	Locality
Carpiliidae	*Carpilius*	*convexus*	594	AF501712	AF501685	Red Sea, Gulf of Aqaba, Elat, Feb. 1959. Coll. Steier. AR-27782, Tel-Aviv University, Zoological Museum. RW01.005.
	Carpilius	*convexus*	595	AF501713	AF501686	Red Sea, Erithrea/Ethiopia, Dahlak Archipelago, 05 April 1962. Coll. L. Fishelson. AR-903886 (E62/3886), Tel-Aviv University, Zoological Museum. RW01.006.
	Carpilius	*convexus*	600	AF501714	AF501687	off Indonesia, on oil rig, collection data unknown. Coll. D.J.W. Lane, det. S.K. Koh. Originally from the Raffles Museum of Biodiversity Research National University of Singapore. ZRC1999.1017. RW01.009.
	Carpilius	*convexus*	S002	AF501716	AF501688	Pacific, off Hawaii, 25°16.124′N, 170°27.745′W, 25.6 m, lobster trap. 25 June 2000. NMFS, SWFC, Honolulu Laboratory, Sta. 362, Cruise TC00-07. Coll. Robert Moffitt. ST00.023.
	Carpilius	*convexus*	S004	AF501717	AF501689	Pacific, off Hawaii, 23°21.241′N, 164°16.109′W, 31–58.5 m, lobster trap. 09 June 2000. National Marine Fisheries Service (NMFS), SWFC, Honolulu Laboratory, Sta. 33, Cruise TC00-07. Coll. Robert Moffitt. ST00.019.
	Carpilius	*convexus*	S005	AF501715	AF501690	Pacific, off Hawaii, 25°37.274′N, 170°48.716′W, 31 m, lobster trap. 28 June 2000. NMFS, SWFC, Honolulu Laboratory, Sta. 430, Cruise TC00-07. Coll. Robert Moffitt. ST00.031.
	Carpilius	*convexus*	S006	AF501718	AF501691	Pacific, off Hawaii, 25°38.221′N, 170°45.911′W, 45.7–47.5 m, lobster trap. 28 June 2000. NMFS, SWFC, Honolulu Laboratory, Sta. 432, Cruise TC00-07. Coll. Robert Moffitt. ST00.032
	Carpilius	*convexus*	S007	AF501719	AF501692	Pacific, off Hawaii, 23°15.658′N, 164°25.543′W, 32.8–42 m, lobster trap. 11 June 2000. NMFS, SWFC, Honolulu Laboratory, Sta. 69, Cruise TC00-07. Coll. Robert Moffitt. ST00.047.
	Carpilius	*convexus*	S017	AF501721	AF501693	Pacific, Guam, Mangilao, University of Guam Marine Lab. May 16, 2000. ST00.054.
	Carpilius	*convexus*	S018	AF503461	AF501694	Pacific, Guam, Mangilao, University of Guam Marine Lab. May 16, 2000. ST00.055.
	Carpilius	*corallinus*	596	AF503462	AF501698	Atlantic, Brazil, Atol das Rocas, ~3°52′S, 33°50′W, on reef at night. 28 October 2000. Coll. P. S. Young, P. C. Paiva, and A. A. Aguiar. RW01.007.
	Carpilius	*corallinus*	S023	AF501728	AF501695	Atlantic, Caribbean, Navassa Island, near Pinnacles, 18°24.819′N, 75°01.325′W, SCUBA, 3–4.6 m, rock rubble broken with hammer, R/V Coral Reef II. 19 March 2000. Coll. R. Wetzer. RW00.059.

	Carpilius	*corallinus*	S016/S026/S027	AF501727	AF501696 AF501697	Atlantic, Caribbean, Navassa Island, near Pinnacles, 18°24.871′N, 75°01.534′W, SCUBA, ~20.4 m, on open rock flat on reef, R/V Coral Reef II. 17 March 2000. Coll. R. Wetzer and G. Hendler. (RW00.038) ST00.051.
	Carpilius	*corallinus*	S008/S009	AF501723 AF501724	AF501699	Atlantic, Caribbean, Guana Island, Long Point, Station 95. 21 July 2000. ST00.052.
	Carpilius	*corallinus*	S010/S011	AF501725 AF501726	AF501700 AF501701	Atlantic, Caribbean, Guana Island, Long Point, Station 95. 21 July 2000. ST00.053.
	Carpilius	*maculatus*	S012	AF501729	AF501702	Pacific, off Hawaii, 25°16.074′N, 170°29.533′W, 27.3 m, lobster trap. 25 June 2000. NMFS, SWFC, Honolulu Laboratory, Sta. 369, Cruise TC00-07. Coll. Robert Moffitt. ST00.024.
	Carpilius	*maculatus*	S014	AF501730	AF501703	Pacific, off Hawaii, 25°16.081′N, 170°29.018′W, 25.5 m, lobster trap. 25 June 2000. NMFS, SWFC, Honolulu Laboratory, Cruise TC00-07, Sta. 367. Coll. Robert Moffitt. ST00.035.
	Carpilius	*maculatus*	S015	AF501731	AF501704	Pacific, off Hawaii, 25°37.426′N, 170°46.670′W, 27.3 m, lobster trap. 28 June 2000. NMFS, SWFC, Honolulu Laboratory, Sta. 424, Cruise TC00-07. Coll. Robert Moffitt. ST00.003.
	Carpilius	*maculatus*	S021	AF501732	AF501705	Pacific, Guam, Mangilao, University of Guam Marine Lab. 16 May 2000. ST00.058.
	Carpilius	*maculatus*	S022	AF501733	AF501706	Pacific, Guam, Mangilao, University of Guam Marine Lab. 16 May 2000. ST00.059.
	Liomera	*cinctimana*	S019	AF501736	AF501708	Pacific, Guam, Mangilao, University of Guam Marine Lab. 16 May 2000. ST00.056.
	Liomera	*cinctimana*	S020	AF501737	AF501707	Pacific, Guam, Mangilao, University of Guam Marine Lab. 16 May 2000. ST00.057.
Xanthidae	*Liagore*	*rubromaculata*	601	AF501734	AF501709	Andaman Sea, Thailand, Phuket, Pichai fishport. 3–6 May 2000. Coll. P.K.L. Ng et al., det. P.K.L. Ng. Originally from the Raffles Museum of Biodiversity Research National University of Singapore. ZRC 2000.0834. Donated to LACM, CR2000042. RW 01.010.
	Liagore	*rubromaculata*	602	AF501735	AF501710	Thailand, Phuket, Pichai fishport. 3–6 May 2000. Coll. P.K.L. Ng et al., det. P.K.L. Ng. Originally from the Raffles Museum of Biodiversity Research National University of Singapore. ZRC 2000.0834. Donated to LACM, CR2000042. RW 01.010.
	Pseudocarcinus	*gigas*	S024	AF501738	AF501711	Australia, Cairns Marine Exports. Specimen purchased. ST00.061.

C. maculatus had one haplotype for each gene fragment (12S- and 16S-rDNA). *C. convexus* had five haplotypes for each gene fragment. *C. corallinus* had five 12S-rDNA haplotypes and one 16S-rDNA haplotype.

AF235983; Galatheoidea *Allopetrolishes angulosus* AF260609; and Palinuroidea *Panulirus gracilis* AF337964, *P. interruptus* AF337959, *Scyllarides nodifer* U96088.

12S-rDNA sequences: Ocypodoidea *Tmethypocoelis ceratophora* AB002127, *Dotilla wichmanni* AB002126, *Scopimera bitympana* AB002125, *S. globosa* AB002124.

DNA was extracted from approximately 0.15–0.20 g of tissue taken from the base of a pereopod. Tissue was macerated with a pestle in a 1.5 ml PCR tube with the buffer provided in the QIAamp Tissue Kit (Qiagen, Valencia, CA) and incubated in a heating block at 55 °C overnight on a shaking table set to medium speed. The extraction protocol followed the manufacturer's instructions. For the PCR 1 μl of DNA template was used in 50 μl PCRs with GibcoBRL Life Technologies (Alameda, CA) 10× buffer and the manufacturer's Platinum *Taq* DNA polymerase (1.25 U). An initial denaturation period of 3 min at 95 °C was followed by 35 cycles at 94 °C for 1 min, annealing at 51 °C for 30 s, and extending for 1 min at 72 °C. 16S- and 12S-rDNA sequences were amplified in both directions using the universal 16Sar and 16Sbr primers (Palumbi et al., 1991) and crustacean specific 12SCRF and 12SCRR primers (Wetzer, 2001) amplifying ~550 and ~430 bp regions, respectively. Six μl of amplified product was electrophoresed on 1% agarose gel and checked for proper size. The remaining PCR product was purified with Sephadex G-50 columns (Sigma Chemical), and DNA was cycle sequenced with DYEnamic ET Terminators (Amersham Pharmacia Biotech) with both strands sequenced on an ABI 377 automated sequencer.

2.2. Data analysis

Nucleotide sequences were edited using the Sequencher software package (version 4.1, GeneCodes, Ann Arbor, MI) and aligned with the sequence alignment program CLUSTAL X 1.81 (Jeanmougin et al., 1998). Default settings were used: pairwise parameters = slow-accurate, gap opening 10.00, gap extension 0.01; multiple parameters = gap opening 10.00, gap extension 0.20, delay divergent sequences 30%, DNA transition weight 0.50. Ingroup taxa were aligned to one another, and the profile alignment option in CLUSTAL X 1.81 was used to align the outgroups (Astacoidea, Galatheoidea, and Palinuroidea) to the ingroup.

The phylogenetic analysis program PAUP* version 4.0b8 (Macintosh) (Swofford, 2001) was used for all parsimony analyses. 16S- and 12S-rDNA datasets were treated as separate data partitions and evaluated with equal weighted parsimony. Subsequent analyses also used six-parameter parsimony (6P) step matrices, calculated according to Cunningham (1997) and Stanger-Hall and Cunningham (1998). In these analyses, transformations were weighted by the negative loga-

rithm of their frequences. All maximum parsimony (MP) analyses were heuristic searches with gaps in nucleotide data treated as missing data. In separate analyses, gaps were treated as a fifth character state. Multistate characters were interpreted as uncertain, all characters were unordered, character-state optimization was based on the accelerated transformation algorithm, and tree robustness was assessed using the bootstrap method (Felsenstein, 1985). One thousand bootstrap replicates based on heuristic searches were run for both the equal weighted and 6P analyses. One hundred random sequence addition heuristic searches in all analyses were performed to identify potential multiple tree islands and determine confidence in the resulting relationships.

A combined 16S-rDNA + 12S-rDNA MP analysis was based on 984 characters, with 514 parsimony informative characters, gaps treated as a fifth character, missing data scored as "?." 16S- and 12S-rDNA sequence data were generated for all specimens sequenced for this project (Table 1). Additional taxa previously published in GenBank (listed in Section 2) were included in this analysis, but for these taxa only 16S-rDNA sequences were available. To reduce analysis time, the topology was partially constrained as follows: swimming crabs (*Ilyoplax*, *Dotilla*, *Scopimera*, and *Callinectes*) and lobsters, (*Panulirus*, *Scyllarides*). No other taxa were constrained. Hundred ten bootstrap replicates based on heuristic searches were run. Only "xanthoid" relationships are shown in Fig. 3.

For the maximum likelihood (ML) analyses the most appropriate model of evolution for the data was obtained by calculating likelihood scores for 56 models of evolution. Modeltest version 3.04 (Posada and Crandall, 1998, 2001) was used to test alternative models of evolution for our data using likelihood-ratio tests. ML analyses were performed using the UNIX version of PAUP* 4.0d65. These models were then statistically compared using a χ^2 test to reject or fail to reject the null hypothesis of DNA substitution as described in Harris et al. (2000). The model with the smallest Akaike value (AIC) served as the model of evolution for the ML analyses. The models of evolution determined most appropriate for the 16S- and 12S-rDNA dataset ML analyses were TrN + I + G ($-\ln L = 7612.66$, AIC = 15239.3) [(TrN, Tamura and Nei, 1993) + (proportion invariable sites = 0.2304) + (gamma distribution = 0.4918)] and GTR + G ($-\ln L = 2270.73$, AIC = 4559.5) [(GTR, Rodríguez et al., 1990) + (gamma distribution shape parameter = 0.4447)], respectively. Nucleotide base frequencies for each dataset are as follows. *16S-rDNA*: $A = 0.3839$, $C = 0.0607$, $G = 0.1409$, and $T = 0.4145$; *12S-rDNA*: $A = 0.3976$, $C = 0.1064$, $G = 0.1414$, and $T = 0.3546$. The ML bootstrap search was constrained by the CPU intensive nature of the analysis, i.e., 100 replicates took 52 days.

To test whether (1) carpiliids + menippids or (2) carpiliids + *Liomera* + *Liagore* are sister taxa, two separate constraint trees were built and tested for compatibility with the optimal MP and ML trees using PAUP*. MP trees were tested using the Kishino–Hasegawa (1989) and Templeton (1983) tests. ML trees were evaluated with the Kishino–Hasegawa (1989) and Shimodaira–Hasegawa (1999) tests.

To test for a constant-rate Poisson distribution process of substitution for this dataset, i.e., molecular clock, we used the likelihood-ratio test (LRT) (Felsenstein, 1988; Goldman, 1993). The 16S-rDNA maximum likelihood phylogeny was estimated using the best fit model and repeated while constraining the estimate to fit the molecular clock model. The LRT statistic was estimated from the formula: $2 \times$ [null hypothesis (= clock enforced) − alternate hypothesis (= clock not enforced)]. Degrees of freedom = number of taxa in dataset − 2.

3. Results

3.1. Sequence divergence

The aligned 16S-rDNA dataset contained 42 sequences (35 species) and 554 characters (289 parsimony informative sites), and the aligned 12S-rDNA dataset included 20 sequences (10 species) and 430 characters (187 parsimony informative sites) (Table 1). Percent-sequence divergence ("uncorrected p," the relationship of the number of aligned sequence positions containing identical residues divided by the number of sequence positions compared) was calculated. Intraspecific variation, i.e., haplotypes and sequence divergences for 16S- and 12S-rDNA gene fragments, is reported in Table 2. A single haplotype was recovered for the 16S-rDNA region sequenced in *C. corallinus* and likewise for *C. maculatus*. Five haplotypes were identified in

C. convexus. For the 12S-rDNA gene fragment, one haplotype was recovered for *C. maculatus*, and five haplotypes each for *C. corallinus* and *C. convexus*. Sequence divergences for interspecific relationships ranged from 5.7 to 10.2% (Table 3).

3.2. Phylogenetic analyses

16S- and 12S-rDNA datasets were used as separate estimates of carpiliid phylogeny. Topologies resulting from separate analyses were fully compatible with Fig. 1. Treating gaps as either missing and or as a fifth character state had no affect on the topology. MP and ML analyses resulted in a single topology in which the two Pacific species, *C. convexus* and *C. maculatus*, are more closely related to each other than either is to the Caribbean species *C. corallinus* (Fig. 1). The Red Sea *C. convexus* individuals form a well-supported clade, as do the Hawaii and Guam individuals. The 16S-rDNA MP analysis produced a single most parsimonious tree, tree length 1714 steps, and CI = 0.352. The MP 6P analysis for the same dataset produced three most parsimonious trees with tree length = 2642 steps and CI = 0.322, which are also compatible with the topology in Fig. 1.

The tree resulting from the 16S-rDNA ML analysis is provided in Fig. 2 and is compatible with Fig. 1. Bootstrap scores for 1000 MP and 100 ML replicates are indicated on the figure. The 12S-rDNA MP analysis produced four most parsimonious trees, 405 steps, and CI = 0.756, all of which are compatible with the consensus tree in Fig. 1 for the 16S-rDNA dataset. In all of our analyses, carpiliids are a monophyletic sister clade to an incompletely resolved polytomy of related crabs: panopeids, xanthids, menippids, trapeziids, ocypodids, and *Pseudocarcinus*. Portunids appear ancestral to this group. Regardless of the crab groups included or excluded from the analyses, the carpiliid relationships remained unchanged. The topology of non-carpiliid taxa

Table 2
Intraspecific variation with haplotypes isolated and sequence divergences for respective gene fragments

	No. of individuals sequenced	No. of haplotypes and sequence divergence	
C. corallinus	5	1	5 (0.25–6.2%)
C. maculatus	5	1	1
C. convexus	10	5 (0.37–6.0%)	5 (0.25–10.0%)

Table 3
Interspecific 16S- and 12S-rDNA sequence divergences

	C. corallinus	*C. convexus*	*C. maculatus*	
C. corallinus	—	**8.9–10.1**%	**9.4**%	
C. convexus	*8.3–10.2%*	—	**5.8–9.4**%	**16S-rDNA**
C. maculatus	*5.7–6.4%*	*7.8–8.4%*	—	
		12S-rDNA		

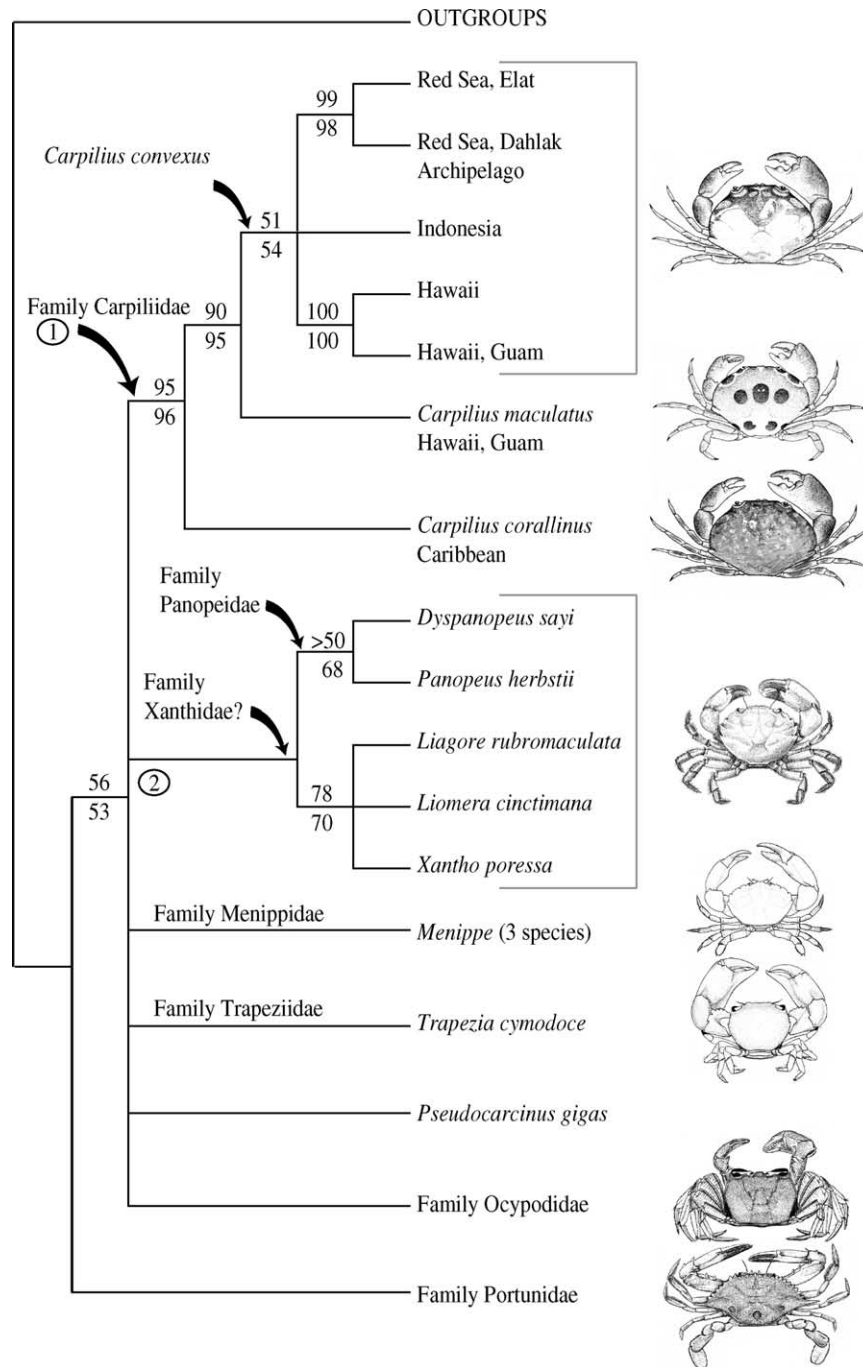16S-rDNA values are indicated in bold, 12S-rDNA values in italics.

Fig. 1. Relationships within the genus *Carpilius* are based on separate 12S- and 16S-rDNA sequence data, and analyses are fully compatible. Values above the branches are the 16S-rDNA bootstrap values for the MP analyses; values below branches are bootstrap values for 6P analyses. The 12S-rDNA analysis was limited to 20 taxa; bootstrap values are not shown. Images are modified after Ng (1998) and Brusca (1980). The branch leading to the figure of the xanthid crab *Xantho* represents the xanthid genera *Liomera*, *Liagore*, *Dyspanopeus*, *Panopeus*, and *Xantho*. The branch leading to the figure of an ocypodid crab (represented here by *Ocypode occidentalis*) represents eight sequences from the genera *Ilyoplax*, *Scopimera*, *Dotilla*, and *Ocypode* (the grapsid *Pachygrapsus transversus* was also in this clade). Similarly, the branch leading to the figure of the portunid crab (represented here by *Portunus*) represents eight portunid sequences from the genera *Callinectes* and *Scylla*. The circled numbers represent possible recognition of the families Carpiliidae (1) and Xanthidae (2). Crabs not drawn to scale.

was dependent on the sequences included and is best depicted as polytomies [(Panopeidae, *Liagore* + *Liomera*, and *Xantho*) and (Carpiliidae, "Xanthidae," Menippidae, Trapeziidae, *Pseudocarcinus*, and Ocypodidae)].

The result of the 16S-rDNA + 12S-rDNA combined MP analyses is provided in Fig. 3. This topology provides increased resolution for the carpiliids and is compatible with the relationships depicted in Figs. 1 and 2.
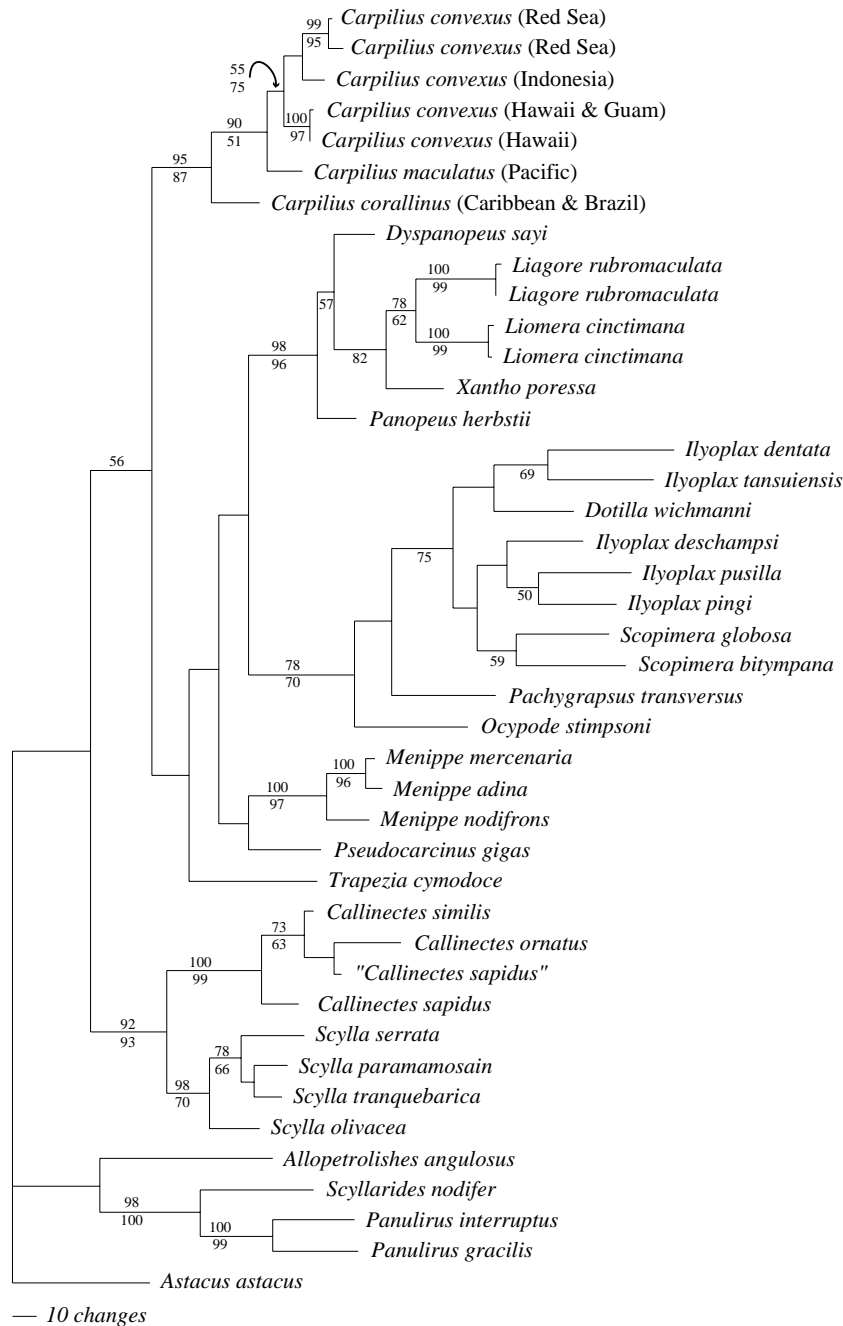
Fig. 2. ML analysis based on 16S-rDNA sequence data, $-\ln L = 8732.45$. Values above the branches are the bootstrap values for the MP analyses (1000 replicates). Values below branches are the bootstrap values for the ML analysis (100 replicates).

Table 4 summarizes the constraint tree comparisons testing two separate hypotheses: (H1) carpiliids + menippids are sister taxa, and (H2) carpiliids + *Liomera* + *Liagore* are sister taxa. The carpiliid + menippid constraint analyses found sixteen most parsimonious trees which were four steps longer than the single most parsimonious tree found in the unconstrained analysis. Statistically, neither the MP nor the ML constraints could reject the null hypotheses, i.e., a relationship between *Carpilius* + *Menippe*. The carpiliid + *Liomera* + *Liagore*

constraint analyses resulted in 16 most parsimonious trees with these topologies 30 steps longer than the most parsimonious tree. Both the ML and MP analyses statistically rejected a carpiliid + *Liomera* + *Liagore* relationship.

The divergences among taxa were tested to determine whether they fit a molecular clock. The $-\ln L$ score for the null hypothesis (= clock enforced) is 7623.91; the $-\ln L$ score for the alternate hypothesis (= clock not enforced) is 7566.82; hence is $2(7623.91 - 7566.82) =$
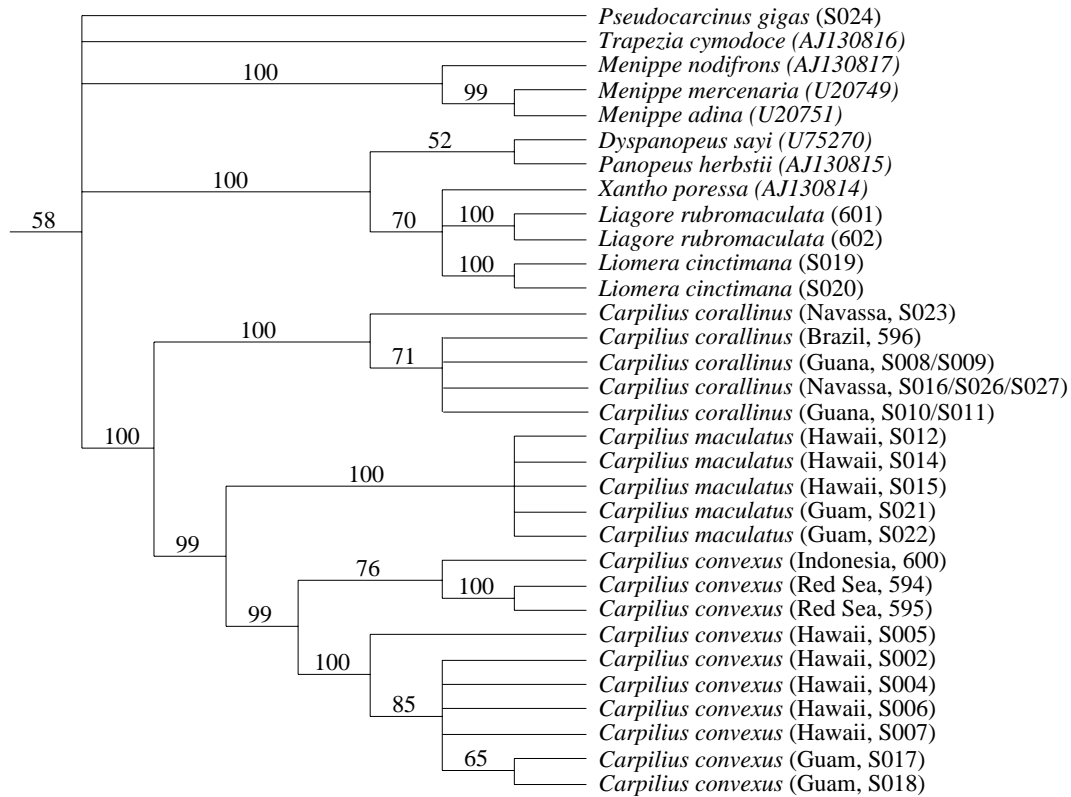
Fig. 3. Relationships of carpiliids based on combined 16S- and 12S-rDNA sequence data. This MP bootstrap analysis is based on 1000 replicates. GenBank numbers are provided for sequences previously published by other researchers and are italicized. Specimens sequenced for this project are denoted with the authors specimen numbers. GenBank numbers for the latter are provided in Table 1.

Table 4
Alternative hypotheses testing

Hypothesis tested	Parsimony (MP)			Likelihood (ML)		
	diff TL	KH	Templeton	$-\ln L$	KH	SH
(H1)	4	0.5558–0.6468	0.8852–1.000	1.654	0.4586	0.446
(H2)	30	0.0001–0.0069*	0.0001–0.0133*	90.473	0.0001*	0.000*

Comparisons of constraint trees corresponding to the two hypotheses: (H1) carpiliids + menippids and (H2) carpiliids + *Liomera* + *Liagore*. For the MP analyses the difference in tree lengths (diff TL) and the probability of getting a more extreme T value under the null hypothesis of no difference for the Kishino–Hasegawa (KH) and Templeton tests are provided. For the ML analyses the difference in the log likelihood score ($-\ln L$) and the probability of getting a more extreme T value under the null hypothesis of no difference for the KH and Shimodaira–Hasegawa (SH) tests are provided. Asterisks (*) indicate significance at $\alpha = 0.05$.

114.18; 40 df; $P < 0.05$. The null hypothesis is rejected, i.e., the rates of substitution vary significantly among the branches. A clock-like model is inappropriate for this dataset.

4. Discussion

4.1. Carpiliid relationships

It is clear from our analyses of relationships that the genus *Carpilius* comprises a distinct clade (1, in Fig. 1) that is not part of the Xanthidae sensu stricto. Thus, our analysis strongly supports the recognition of the Carpiliidae as a monophyletic family of "xanthoid" crabs. It is also clear that the genera *Liagore* and *Liomera* are not as closely related to *Carpilius* as once thought, and there is no support for their inclusion in the family Carpiliidae or for Alcock's (1898) "Alliance Carpilioida." Alternative hypothesis tests rejected a carpiliid + *Liomera* + *Liagore* relationship (Table 4). An affinity between *Carpilius* and *Menippe*, previously posited based on the number of larval stages, could not be discounted. The three species of *Menippe* never clustered with *Carpilius* in the MP and ML analyses. However, alternative hypothesis testing could not reject a carpiliid + menippid

relationship (Table 4). Additional taxa would have to be sequenced to test the composition of the Carpiliidae as suggested by Schweitzer (2000) (i.e., whether *Euryozius* is closely related to *Carpilius*).

Carpilius corallinus appeared basal to the two Pacific species in every analysis. The two Pacific species, *C. convexus* and *C. maculatus*, are closely related, as might be expected. There is geographic structure within *C. convexus* individuals surveyed. However, since this is not a population study, too few individuals were surveyed to gain insight into population structure. Specimens of *C. convexus* from the Red Sea differ from those from Hawaii, but not enough to question the validity of the species, and far less than the difference between any *C. convexus* specimens and *C. maculatus*. Thus, the argument that *C. convexus* and *C. maculatus* might be different color morphs of a single species (Paul'son, 1961) is clearly unsupported.

The relationships of carpiliids to other xanthoid crabs and to other brachyuran families is less clear. The claim by Alcock (1898) that the genera *Liomera* and *Liagore* are closely allied with *Carpilius* is unsupported. In our analysis, *Liagore* and *Liomera* are more closely related to *Xantho* and to panopeids (*Dyspanopeus* and *Panopeus*) than either genus is to *Carpilius*. There is weak bootstrap support (56 and 53%) for an assemblage that could be recognized as the "Xanthoidea," (sensu Martin and Davis, 2001) though it also would include the Ocypodidae (2, in Fig. 1), which seems unlikely to us. The restricted Xanthidae (for which bootstrap support is less than 50% in MP and 68% in 6P analyses, respectively), would include the Panopeidae as currently recognized (Martin and Davis, 2001) plus *Liagore*, *Liomera*, *Xantho*, and presumably many more xanthid genera. The giant Tasmanian crab *P. gigas* and the obligate coral crabs (*Trapezia*), both treated historically as "xanthids," appeared no closer to *Carpilius* than did other groups (e.g., Ocypodidae) (Fig. 1).

4.2. Outgroup selection and its effect on phylogenetic relationships

The relationships of *Carpilius* species and the family Carpiliidae are robust. These relationships were maintained regardless of gene choice, taxa included in analyses, or number of outgroups used. However, outgroup choice did affect the placement of carpiliids in relation to other crab groups. At present the best phylogenetic representation of the taxa included in this study is an unresolved polytomy of the Carpiliidae, "Xanthidae," Menippidae, Trapeziidae, Ocypodidae, and *Pseudocarcinus*. Regardless of the species of panopeids included in the analyses, these were always a monophyletic clade. Similarly, menippids are monophyletic. The single *Trapezia* sequence in this analysis is an extremely long branch requiring additional sampling at the family level. Finally,

a broader and more extensive survey of "Xanthidae" genera in future studies will be necessary to shed light on the phylogenetic relationships of xanthoids. We found the resolving power of the 16S- and 12S-rDNA markers appropriate for this study and the questions posed (i.e., relationship within *Carpilius*). However, a future study at the "xanthoid" level would likely benefit from using a more slowly evolving nuclear molecular marker (e.g., 18S-rDNA, elongation factor 1α).

4.3. Biogeography and timing of separation

The earliest known occurrences of members of the family Carpiliidae are fossils attributed to the genera *Palaeocarpilius* from Europe, India, and Egypt found in middle to upper Eocene (55.6 mybp) rocks and *Harpactoxanthopsis* of Europe by Schweitzer (2000). Schweitzer et al. (2000) and Schweitzer (2000) considered specimens from the Eocene (55.6 mybp) in Washington to be the earliest known members of *Carpilius*. *Carpilius* is also known from the early middle Miocene of southwest Japan (Karasawa, 1993), the Pliocene of Barbados (Collins and Morris, 1976), and the Pleistocene–Holocene of Taiwan (Hu and Tao, 1996). The most recent fossils are known from the late Pleistocene of Jamaica (Collins et al., 1996). No extant species of *Carpilius* are known from the eastern Pacific or eastern Atlantic.

This fossil record, while better than for many other crustacean taxa, is inadequate to calibrate a molecular clock for the group. In instances of a poor or nonexistent fossil record, geological events rather than first appearances of sister-taxa are most commonly used. For 16S-rDNA sequence data, Sturmbauer et al. (1996) estimated a sequence divergence rate of 0.9% per million years for fiddler crab populations (*Uca*) across the Isthmus of Panama (closure 3.1–3.5 mybp). Schubart et al. (1998) estimated a slightly lower rate of 0.65–0.88% per million years for trans-isthmian grapsid crabs (*Sesarma*). More recently, Wares (2001) estimated the rate of evolution at 0.67% divergence per million years for chthamalid barnacles calibrated to the rising Isthmus of Panama and the opening of the Sea of Cortez. Extrapolations of our data using the extremes of these evolutionary rates for crustaceans (0.65–0.9% per million years) result in estimated divergences for *C. convexus* and *C. maculatus* of 6.4–14.5 million years and for *C. corallinus* and *C. maculatus* of 10.4–14.5 million years. It is important to note that this type of extrapolation is only an approximation of divergence time. Our LRT found substitution rates to vary significantly among branches. Hence these dates are regarded as "ballpark" estimates only.

Schweitzer (2000) hypothesized, based on the fossil record, that the family Carpiliidae arose in the Tethyan region during the Eocene and spread westward to both coasts of North America via Atlantic Ocean surface

currents and the Straits of Panama. Yet she also proposed that the European members of the family could have spread eastward to the Pacific Ocean via the Tethys Sea. Schweitzer (2000) stated that the early appearance of the family on the eastern and western coasts of North America suggests that the Carpiliidae reached the eastern Pacific Ocean (where the family is not found today) via the Straits of Panama. In particular, she proposed that the genus *Carpilius* arose in the North Pacific Ocean during the Eocene and subsequently dispersed throughout the Pacific by ocean surface currents and into the Caribbean and equatorial Atlantic Ocean via the Straits of Panama. Our results suggest that the radiation may have been in the opposite direction (Caribbean to Pacific).

Acknowledgments

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