The Decapod Tree of Life: Compiling the Data and Moving toward a Consensus of Decapod Evolution

Heather D. Bracken [1]*, Alicia Toon [1], Darryl L. Felder [2], Joel W. Martin [3], Maegan Finley [1], Jennifer Rasmussen [1], Ferran Palero [4] & Keith A. Crandall [1]

[1] Department of Biology, Brigham Young University, Provo, UT, USA
[heather.bracken@gmail.com], [a.toon@griffith.edu.au], [mjfin22@gmail.com],
[jennmras@gmail.com], [keith_crandall@byu.edu]

[2] Department of Biology, University of Louisiana, Lafayette, LA, USA
[dlf4517@louisiana.edu]

[3] Natural History Museum of Los Angeles County, Los Angeles, CA, USA
[jmartin@nhm.org]

[4] Departament de Genètica, Facultat de Biologia, Universitat de Barcelona,
Av. Diagonal 645, 08028 Barcelona, Spain
[ferranpalero@ub.edu]

* Corresponding author

> Abstract

The order Decapoda represents a species-rich group of crustaceans. Numerous economically important and morphologically diverse members of this group have been studied extensively for many decades, in part to understand their phylogeny. There are several competing hypotheses concerning relationships among the major lineages of Decapoda. Our laboratories are estimating a robust decapod phylogeny based on molecular and morphological data in an attempt to resolve relationships among major lineages. The order includes roughly 175 families and more than 15,000 described species (extant and extinct). Interpretations are complicated by the estimated 437 million years since origin of the Decapoda, with all the major lineages likely established by 325 million years ago. Constructing a molecular phylogeny across such a timescale requires markers with enough variation to infer relationships at the fine scale (at and within the family level) but which are conservative enough to reflect deeper divergences across infraorders. Here we present a molecular phylogeny for the order Decapoda, combining nuclear and mitochondrial sequences, to investigate relationships among nine pleocyemate infraorders, one dendrobranchiate superfamily, 56 families, 113 genera, and 128 species. New and available sequence data are assembled to build the most extensive decapod phylogeny to date both in terms of taxon representation and genetic coverage. We discuss current and new hypotheses of decapod relationships and suggest a plan for the movement towards a consensus of decapod evolution.

1. Introduction

Decapods – including crabs, shrimps, lobsters and crayfish – are among the most species-rich groups of crustaceans, representing approximately 175 families and 15,000 described taxa (extant and extinct). They are economically important, bringing in billions of dollars each year to world fisheries. The economic importance of this group, together with their distinctive morphology and ecological diversity, makes decapod crustaceans popular research subjects in all fields of biology.

Despite widespread interest in this group, phylogenetic relationships among decapods at many taxonomic levels remain unsettled. Complicating interpretation of these lineages is the estimated 437 million years since the origin of the Decapoda (PORTER et al. 2005) coupled with the group's morphological diver-
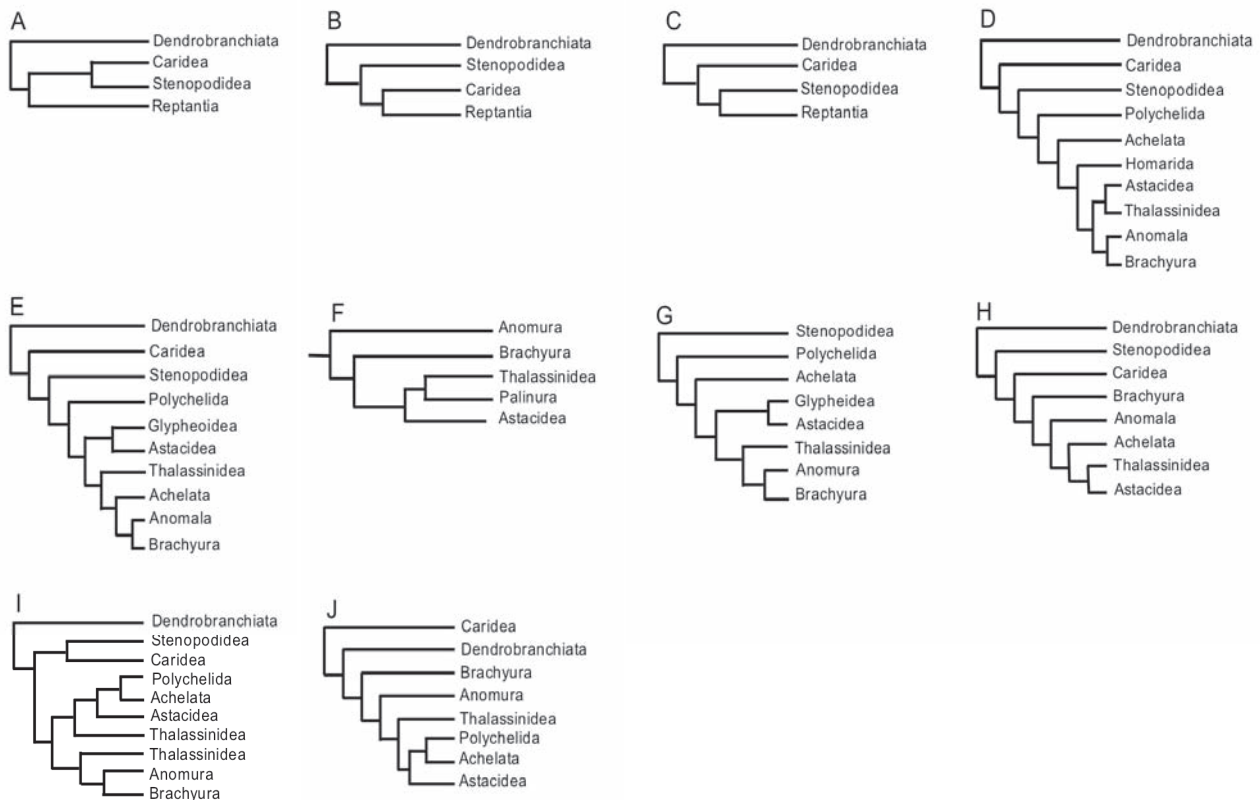
Fig. 1. Hypotheses of higher-level decapod relationships based on morphological similarity (A–C); morphological cladistic analyses (D, E); and molecular phylogenetic analyses (F–J). **A**: Burkenroad (1963, 1981); **B**: Christoffersen (1988); **C**: Abele & Felgenhauer (1986); **D**: Scholtz & Richter (1995); **E**: Dixon et al. (2003); **F**: Crandall et al. (2000); **G**: Ahyong & O'Meally (2004); **H**: Porter et al. (2005); **I**: Tsang et al. (2007); **J**: Toon et al. (2009).

sity and complexity. Inferring phylogenetic relationships across such a broad timescale and species-rich group has resulted in many conflicting hypotheses of decapod evolution (Fig. 1).

Early classifications divided the decapods into swimming (Natantia) and walking (Reptantia) lineages (Boas 1880). However, later findings based largely on gill morphology and reproductive biology proposed a new classification for the Decapoda and rendered the Natantia paraphyletic (Burkenroad 1963, 1981). Today, there is little debate over the monophyly of the major decapod suborders Dendrobranchiata and Pleocyemata. Likewise, the basal position of the informal "natant" groups (Caridea, Penaeoidea, and Stenopodidea) is generally accepted, and many studies have recovered the traditional "reptant" groups (Brachyura, Anomura, Thalassinidea, Astacidea, Palinura) as a monophyletic clade (Crandall et al. 2000; Schram 2001; Ahyong & O'Meally 2004; Porter et al. 2005; Tsang et al. 2008b; Robles et al. 2009; Toon et al. 2009). However, hypotheses concerning the internal relationships at the infraordinal and family levels are dynamic and under continuous debate.

Conflicting hypotheses arise in morphological and molecular studies of the Decapoda (Fig. 1) (Burken-road 1963, 1981; Abele & Felgenhauer 1986; Christoffersen 1988; Scholtz & Richter 1995; Dixon et al. 2003; Ahyong & O'Meally 2004; Porter et al. 2005; Tsang et al. 2008b; Toon et al. 2009). Generating a suite of phylogenetically informative characters across this diverse and complex group has proved daunting for morphological cladists. Partly for this reason, molecular analyses have provided an attractive method for addressing decapod phylogeny in recent years. However, different combinations of conservative and variable molecular markers, both mitochondrial and nuclear, have resulted in conflicting phylogenies depending on gene and taxon selection. For example, five recent molecular studies have proposed five different reconstructions of "reptant" relationships, all of which used a different number and combination of genes (Fig. 1). Similar contradictions have been found in attempts to use morphological data to address the specific position of "natant" lineages in relation to the remaining decapods (Fig. 1).

The continuing quest to find new genes with which to build new phylogenies may, at present, be hindering our ability to draw consensus from currently available molecular data. While we acknowledge the merit of continued search for phylogenetically informative

markers, it is important that we add these new genes to growing datasets and not use them in isolation. We herewith move toward a consensus analysis based upon a set of genetic markers (H3, 18S, 28S, 16S) that have been applied to the decapod crustaceans over years of molecular research. As we accumulate more taxa and phylogenetically informative markers, this will be a dataset to build upon. New and available sequence data are here merged to establish a "milepost" in our ongoing studies of decapod evolution.

The Decapod Tree of Life Project (see http://deca poda.nhm.org/) is charged to reach for such consensus in an attempt to resolve higher-level relationships within decapod crustaceans while also reconstructing the origins and evolution of this ancient group. Here we present a molecular phylogeny for the order Decapoda, combining nuclear and mitochondrial sequences, to investigate the relationships among nine pleocyemate infraorders, one dendrobranchiate superfamily, 56 families, 113 genera, and 128 species. In combination, the new and available sequence data here assembled build the most extensive decapod phylogeny to date both in terms of taxon representation and number of characters used to estimate relationships. Our study highlights monophyletic and polyphyletic assemblages, while also examining congruence or incongruence between past and present hypotheses.

2.　Materials and methods

2.1.　Ingroup taxa and outgroup selection

Decapod representatives from all pleocyemate infraorders and dendrobranchiate superfamilies (excluding Sergestoidea), 56 families, 113 genera, and 128 species were included in the analysis (Tab. 1). Morphological and molecular evidence has shown the infraorder Palinura to be polyphyletic (SCHOLTZ & RICHTER 1995; SCHRAM 2001; DIXON et al. 2003; AHYONG & O'MEALLY 2004). Past studies have divided the group into separate infraorders including Achelata, Polychelida and Glypheidea (SCHOLTZ & RICHTER 1995; DIXON et al. 2003; AHYONG & O'MEALLY 2004), which we herein follow as our frame of reference (Tab. 1). Since we are interested in infraordinal and family-level relationships, exemplars from decapod families were chosen based on sequence availability. We attempted to sample more extensively within taxonomically diverse and problematic groups. Most sequences were obtained from GenBank or from collaborators, and new sequences are highlighted in bold (Tab. 1) and have been deposited in GenBank.

Initially we included a variety of outgroup taxa from the eumalacostracan orders Isopoda, Amphipoda, Hoplocarida, and Euphausiacea with hopes to better resolve the relationships within decapod crustaceans (Tab. 1). However, since the inclusion of isopods and amphipods resulted in highly divergent and poorly aligned positions within individual alignments, we excluded these taxa from the final analyses. Sequences representing the putative sister order Amphionidacea were not available for inclusion in the analysis. All outgroup sequences were obtained from GenBank.

2.2.　Gene selection

The goal of this study was to include a robust array of taxa while sampling across an adequate number of molecular markers. With the origin of the Decapoda estimated at 437 million years, we were forced to choose markers that resolve relationships across a broad timescale. For these reasons, one mitochondrial gene (16S) and three nuclear genes (18S, 28S, H3) were selected. Past studies have shown these genes' utility in resolving phylogenetic relationships at different taxonomic levels and time scales (SPEARS et al. 1992, 1994; GIRIBET et al. 1996; SCHUBART et al. 2000; STILLMAN & REEB 2001; TUDGE & CUNNINGHAM 2002; PORTER et al. 2005; MANTELATTO et al. 2006, 2007; ROBLES et al. 2007). Any increase in taxon sampling decreased the number of genes that could be utilized for phylogenetic reconstruction, and any increase in gene selection severely lowered the number of taxa that could be included in the analysis. Our group continues to expand both our taxon sampling and the genetic sampling for addressing decapod relationships.

2.3.　DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from the abdomen, gills, pereopods or pleopods using the Qiagen DNeasy® Blood and Tissue Kit (Cat. No. 69582). Targeted gene regions were amplified by means of the polymerase chain reaction (PCR) using one or more sets of primers. The 16S large ribosomal subunit (~550 bps, CRANDALL & FITZPATRICK 1996) was selected as the mitochondrial gene, and the 18S small ribosomal subunit (~1900 bps, WHITING et al. 1997; WHITING 2002), 28S large ribosomal subunit (~2500 bps, WHITING et al. 1997; WHITING 2002; TOON et al. 2009), and H3 protein-coding gene (~330 bps, COLGAN et al. 1998) were selected as the nuclear genes.

Reactions were performed in 25 μl volumes containing 0.5 μM forward and reverse primer for each gene, 200 μM each dNTP, PCR buffer (with magnesium chloride), 1 unit HotMasterTaq polymerase

Tab. 1. Taxonomy, voucher catalog numbers, and GenBank accession numbers for gene sequences used in this study. An "—" designates missing sequence data and an "N/A" indicates unavailable voucher numbers. * = Excluded from final analysis. New sequences are indicated in bold. All others were obtained from GenBank or collaborators.

Taxon	Voucher	GenBank Nos. 16S	GenBank Nos. 18S	GenBank Nos. 28S	GenBank Nos. H3
OUTGROUP TAXA					
Euphausiacea Dana, 1852					
Euphausiidae Dana, 1852					
Euphausia eximia Hansen, 1911	KACeuex	DQ079713	DQ79748	DQ079787	DQ079674
Nematoscelis sp.	KACnesp	DQ079725	DQ79760	DQ079801	DQ079690
Hoplocarida Calman, 1904					
Stomatopoda Latreille, 1817					
Lysiosquillidae Giesbrecht, 1910					
Lysiosquillina maculata (Fabricius, 1793)	KC3832	EU920935	EU920967	EU920998	EU921076
Squillidae Latreille, 1802					
Kempina mikado (Kemp & Chopra, 1921)	EBU39264	—	AF370802	EU289819	AF110873
Squilla empusa Say, 1818	N/A	AF107617	L81946	AY210842	—
Peracarida Calman 1904					
Amphipoda Latreille, 1816					
Gammaridae Latreille, 1802					
Gammarus lacustris Sars, 1863*	323	AY926724	EF582915	EF582964	—
Isopoda Latreille, 1817					
Asellidae Latreille, 1802					
Asellus aquaticus (Linnaeus, 1758)*	N/A	AF532161	AJ287055	AY739195	—
Munnopsidae Lilljeborg, 1864					
Acanthocope galathea Wolff, 1962*	N/A	—	AF496656	EF682337	—
INGROUP TAXA					
Decapoda Latreille, 1802					
Dendrobranchiata Bate, 1888					
Penaeoidea Rafinesque, 1815					
Penaeidae Rafinesque, 1815					
Farfantepenaeus duorarum (Burkenroad, 1939)	KC4282	**FJ943438**	**FJ943445**	**FJ943451**	**FJ943459**
Penaeus semisulcatus de Hann, 1844	KC1269	DQ079731	DQ079766	DQ079809	DQ079698
Penaeus sp.	Kcpen	EU920934	EU920969	EU921005-EU921006	EU921075
Pleocyemata Burkenroad, 1963					
Stenopodidea Claus, 1872					
Stenopodidae Claus, 1872					
Stenopus hispidus (Olivier, 1811)	MLP119	DQ79734	DQ079769	DQ079812	DQ079701
Stenopus hispidus (Olivier, 1811)	KC4276	**FJ943437**	**FJ943443**	**FJ943450**	**FJ943457**
Spongicolidae Schram, 1986					
Microprosthema inornatum Manning & Chace, 1990	KC4278	—	**FJ943444**	**FJ943452**	**FJ943458**
Caridea Dana, 1852					
Alpheidae Rafinesque, 1815					
Betaeus harrimani Rathbun, 1904	KC3103	**FJ943434**	**FJ943440**	**FJ943447**	**FJ943454**
Metabetaeus sp.	KC3109	**FJ943435**	**FJ943441**	**FJ943448**	**FJ943455**
Anchistioididae Borradaile, 1915					
Anchistioides antiguensis (Schmitt, 1924)	KC3051	EU920911	EU920936	EU920971	EU921043
Atyidae de Haan, 1849					
Atyoida bisulcata (Randall, 1840)	KC2138	DQ079704	DQ079738	DQ079774	DQ079661
Typhlatya pearsei Creaser, 1936	MLP85.1	DQ079735	DQ079770	DQ079813	DQ079702
Crangonidae Haworth, 1825					
Crangon crangon (Linnaeus, 1758)	KC3052	EU920915	EU920938	EU920972	EU921047
Disciadidae Rathbun, 1902					
Discias sp.	KC3108	EU920921	EU920941	EU920986	EU921054
Hippolytidae Dana, 1852					
Eualus gaimardii (H. Milne Edwards, 1837)	KC3056	EU920923	EU920940	EU920973	EU921057
Hippolyte bifidirostris Miers, 1876	KC3059	EU920927	EU920939	EU920974	EU921063
Lysmata debelius (Bruce, 1983)	MLP121	DQ079718	DQ079752	DQ079793	DQ079681
Lysmata wurdemanni (Gibbes, 1850)	MLP120	DQ079719	DQ079753	DQ079794	DQ079682
Palaemonidae Rafinesque, 1815					
Coutierella tonkinensis Sollaud, 1914	KC3068	EU920920	EU920937	EU920975	EU921053
Creaseria morleyi (Creaser, 1936)	MLP102.1	DQ079710	DQ079746	DQ079784	DQ079671
Cryphiops caementarius (Molina, 1782)	JC1219	DQ079711	DQ079747	DQ079785	DQ079672
Macrobrachium potiuna (Müller, 1880)	KC2094	DQ079721	DQ079756	DQ079797	QO79685
Macrobrachium sp.	MLP123.2	DQ079720	DQ079754	DQ079795	DQ079683

Tab. 1. Continued (ingroup taxa).

Taxon	Voucher	GenBank Nos. 16S	GenBank Nos. 18S	GenBank Nos. 28S	GenBank Nos. H3
Palaemon elegans Rathke, 1837	KACpael	DQ079729	DQ079764	DQ079807	DQ079696
Palaemonetes paludosus (Gibbes, 1850)	MLP124	—	DQ079755	DQ079796	DQ079684
Processidae Ortmann, 1890					
Nikoides danae Paulson, 1875	KC3114	**FJ943436**	**FJ943442**	**FJ943449**	**FJ943456**
Polychelida De Haan, 1841					
Polychelidae Wood-Mason, 1874					
Polycheles aculeatus Galil, 2000	AMSEBU-14573	AY583885	AY583959	AY583977	—
Polycheles suhmi Bate, 1978	AMSEBU-14574	AY583887	AY583961	AY583979	—
Polycheles typhlops C. Heller, 1862	KC3101	EU920932	EU920950	EU921003-EU921004	EU921073
Polycheles typhlops C. Heller, 1862	N/A	FJ174890	FJ174921	EU449507	FJ174856
Achelata Scholtz & Richter, 1995					
Palinuridae Latreille, 1802					
Jasus edwardsii (Hutton, 1875)	KC725 / KC3209	DQ079716	AF235972	DQ079791	EU921064
Jasus (Sagmariasus) verreauxii (H. Milne Edwards, 1851)	N/A	FJ174896	FJ174933	FJ174820 FJ174833	FJ174870
Justitia longimana (H. Milne Edwards, 1837)	N/A	AF502953	AF498674	FJ174841	FJ174873
Linuparus trigonus (von Siebold, 1824)	N/A	AF502946	AF498675	FJ174808 FJ174830	FJ174874
Palibythus magnificus Davie, 1990	N/A	AF502950	AF498666	FJ036957 FJ174825 FJ174843	FJ174875
Palinurellus wieneckii (de Man, 1881)	ZRC1992-.8058	AY583889	AY583963	AY583981	—
Palinurus elephas (Fabricius, 1787)	KC3210	EU920929	EU920959	EU920999 EU921000	EU921069
Palinurus barbarae (Groeneveld, Griffiths & van Dalsen, 2006)	N/A	FJ174903	FJ174925	FJ036949 FJ174817 FJ174849	FJ174876
Palinurus mauritanicus Gruvel, 1911	N/A	FJ174901	FJ174923	FJ036945 FJ174813 EU449506	FJ174880
Palinustus waguensis Kubo, 1963	N/A	AF502952	AF498667	FJ174826 FJ174847	FJ174866
Panulirus regius De Brito Capello, 1864	N/A	FJ174899	FJ174916	FJ036938 FJ174802 FJ174827	FJ174883
Panulirus regius De Brito Capello, 1864	KC2167	DQ079730	DQ079765	DQ079808	DQ079697
Projasus parkeri (Stebbing, 1902)	N/A	FJ174898	FJ174935	FJ036944 FJ174819 FJ174835	FJ174872
Puerulus angulatus (Bate, 1888)	N/A	AF502951	AF498668	FJ174811 FJ174845	FJ174882
Scyllaridae Latreille, 1825					
Parribacus antarcticus (Lund, 1793)	N/A	FJ174913	AF498676	FJ174822 FJ174832	—
Scyllarides herklotsii (Herklots, 1851)	N/A	FJ174906	FJ174939	FJ036958 FJ174823 FJ174842	FJ174863
Scyllarus arctus (Linnaeus, 1758)	KC2159	DQ079732	DQ079767	DQ079810	DQ079699
Scyllarus caparti Holthuis, 1952	N/A	FJ174909	FJ174928	FJ036953 FJ174806 FJ174839	FJ174860
Thenus unimaculatus (Burton & Davie, 2007)	N/A	FJ174915	FJ174942	FJ036952 FJ174810 FJ174838	FJ174858
Glypheidea Winkler, 1883					
Glypheidae Winkler, 1883					
Neoglyphea inopinata Forest & de Saint Laurent, 1975	NTMCr00-6300	AY583894	AY583968	AY583986	—
Anomura MacLeay, 1838					
Aeglidae Dana, 1852					
Aegla abtao Schmitt, 1942	KAC-Aa4	AY050067	AF439390	AY595965	DQ079658
Aegla alacalufi Jara & Lopez 1981	KAC798	AY050058	EU920958	AY595958	EU921042
Aegla papudo Schmitt, 1942	KAC-A7 KACa0007	AY050032	AY595796	AY595930	—
Albuneidae Stimpson, 1858					
Lepidopa californica Efford, 1971	N/A	AF436054	AF436015	AF435996	—
Blepharipodidae Boyko, 2002					
Blepharipoda occidentalis Randall, 1840	N/A	AF436053	AF436014	AF435994	—

Tab. 1. Continued (ingroup taxa).

Taxon	Voucher	GenBank Nos. 16S	GenBank Nos. 18S	GenBank Nos. 28S	GenBank Nos. H3
Chirostylidae Ortmann, 1892					
Eumunida funambulus Gordon, 1930	KC3100	EU920922	EU920957	EU920984	EU921056
Uroptychus parvulus (Henderson, 1885)	KACurpa	AY595926	AF439386	AY596097	DQ079703
Coenobitidae Dana, 1851					
Coenobita compressus H. Milne Edwards, 1837	N/A	AF436059	AF436023	AF435999	—
Diogenidae Ortmann, 1892					
Calcinus obscurus Stimpson, 1859	N/A	AF436058	AF436022	AF435998	—
Galatheidae Samouelle, 1819					
Munida subrugosa (White, 1847)	KACmusu	AY050075	AF439382	AY596099	DQ079688
Munidopsis rostrata (A. Milne-Edwards, 1880)	KC3102	EU920928	EU920961	EU920985	EU921066
Hippidae Latreille, 1825					
Emerita brasiliensis Schmitt, 1935	KACembr	DQ079712	AF439384	DQ079786	DQ079673
Kiwaidae Macpherson, Jones & Segonzac, 2005					
Kiwa hirsuta Macpherson, Jones & Segonzac, 2006	KC3116	—	EU920942	EU920987	EU921065
Lithodidae Samouelle, 1819					
Lithodes santolla (Molina, 1792)	LAClisa	AY595927	AF439385	AY596100	DQ079679
Lomisidae Bouvier, 1895					
Lomis hirta (Lamarck, 1818)	KAClohi	AY595928	AF436013	AY596101	DQ079680
Porcellanidae Haworth, 1825					
Petrolisthes armatus (Gibbes, 1850)	N/A	AF436049	AF436009	AF435989	—
Pylochelidae Bate, 1888					
Pomatocheles jeffreysii	KC3097	EU920930	EU920965	EU920983	EU921070
Astacidea Latreille, 1802					
Astacidae Latreille, 1802					
Astacus astacus (Linnaeus, 1758)	JF134	AF235983	AF235959	DQ079773	DQ079660
Pacifastacus leniusculus (Dana, 1852)	JF64	AF235985	AF235961	DQ079806	DQ079695
Cambaridae Hobbs, 1942					
Barbicambarus cornutus (Faxon, 1884)	KC1941	EU920913	EU920951	EU920993	EU921045
Cambarellus shufeldtii (Faxon, 1884)	KC1210	AF235986	AF235962	DQ079778	DQ079665
Cambaroides japonicus (de Haan, 1841)	KC695	AF235987	DQ079742	DQ079779	DQ079666
Cambarus maculatus Hobbs & Pflieger, 1988	KC74	AF235988	AF235964	DQ079780	DQ079667
Orconectes virilis (Hagen, 1870)	JC897	AF235989	AF235965	DQ079804	DQ079693
Procambarus clarkii (Girard, 1852)	KC1497	AF235989	AF235965	DQ079804	DQ079693
Nephropidae Dana, 1852					
Acanthacaris caeca (A. Milne-Edwards, 1881)	KC1877	—	DQ079736	DQ079771	—
Homarus americanus H. Milne Edwards, 1837	KAChoam	HAU11238	AF235971	DQ079788	DQ079675
Homarus gammarus (Linnaeus, 1758)	KC2162	DQ079714	DQ079749	DQ079789	DQ079676
Nephrops norvegicus (Linnaeus, 1758)	KC2163	DQ079726	DQ079762	DQ079803	DQ079692
Nephrops norvegicus (Linnaeus, 1758)	N/A	FJ174889	FJ174918	FJ036942, FJ174804, EU449504	FJ174855
Nephropsis aculeata S.I. Smith, 1881	KC2117	DQ079727	DQ079761	DQ079802	DQ079691
Parastacidae Huxley, 1879					
Astacoides betsileoensis Petit, 1923	KC1822	EU920912	EU920955	EU920992	EU921044
Astacopsis gouldi Clark, 1936	KC1883	AF135969	DQ079737	DQ079772	DQ079659
Cherax cuspidatus Riek, 1969	KC1175	DQ006550	EU920960	EU920996	EU921048
Cherax glaber Riek, 1967	KACchgl	AF135978	DQ079745	DQ079783	DQ079670
Euastacus eungella Morgan, 1988	KC2671	DQ006593	EU920964	EU921001-EU921002	EU921055
Euastacus robertsi Monroe, l977	KC2781	DQ006633	EU920962	EU920988	EU921058
Euastacus spinichelatus Morgan 1997	KC2631	DQ006638	EU920963	EU920989	EU921059
Gramastacus insolitus Riek, 1972	KC640	EU920926	EU920968	EU920994	EU921062
Paranephrops planifrons White, 1842	KC2741				
Ombrastacoides huonensis Hansen & Richardson, 2006	KC611	AF135997	EU920956	EU920995	EU921072
Parastacus defossus Faxon 1898	KC1515	AF175243	EU920953	EU920991	EU921068
Parastacus varicosus Faxon, 1898	KC1529	EU920933	EU920954	EU920990	EU921074
Samastacus spinifrons (Philippi, 1882)	KC1450	AF175241	EU921131	EU921137	—
Virilastacus araucanius (Faxon, 1914)	KC1415	AF175235	AF235970	—	**FJ948189**
Virilastacus araucanius (Faxon, 1914)	KC1416	—	—	FJ966042	—
Thaumastochelidae Bate, 1888					
Thaumastochelopsis sp.	QMW25868	AY583893	AY583967	AY583985	—
Enoplometopidae de Saint Laurent, 1988					
*Enoplometopus occidentalis** (Randall, 1840)	AMSEBU-36368	AY583892	AY583966	AY583984	—
Brachyura Latreille, 1802					
Calappidae Milne Edwards, 1837					
Calappa gallus (Herbst, 1803)	KC3083	EU920917	EU920943	EU920976	EU921050
Cycloes granulosa De Haan, 1837	KC3082	EU920917	EU920943	EU920976	EU921050

Tab. 1. Continued (ingroup taxa).

Taxon	Voucher	GenBank Nos. 16S	GenBank Nos. 18S	GenBank Nos. 28S	GenBank Nos. H3
Cancridae Latreille, 1802					
Cancer pagurus Linnaeus, 1758	KC2158	DQ079708	DQ079743	DQ079781	DQ079668
Dorippidae MacLeay, 1838					
Ethusa sp.	KC3088	EU920925	EU920966	EU920980	EU921061
Dromiidae De Haan, 1833					
Lauridromia dehaani (Rathbun, 1923)*	AMSP67928	AY583899	AY583972	AY583991	—
Goneplacidae MacLeay, 1838					
Carcinoplax suruguensis Rathbun, 1932	KC3087	**FJ943433**	**FJ943439**	**FJ943446**	**FJ943453**
Grapsidae MacLeay, 1838					
Cyclograpsus cinereus Dana, 1851	KC3417	EU920914	EU920945	EU920997	EU921046
Pachygrapsus marmoratus (Fabricius, 1787)	KACpama	DQ079728	DQ079763	DQ079805	DQ079694
Hepatidae Stimpson, 1871					
Hepatus epheliticus (Linnaeus, 1763)	N/A	AF436043	AF436004	AF435984	—
Homolidae De Haan, 1839					
Paramola japonica Parisi, 1915	ZRC1998.461	AY583990	AY583973	AY583992	—
Leucosiidae Samouelle, 1819					
Ebalia tuberculosa (A. Milne-Edwards, 1873)	KC3085	EU920924	EU920944	EU920978	EU921060
Praebebalia longidactyla (Yokoya, 1933)	KC3086	EU920931	EU920946	EU920979	EU921071
Majidae Samouelle, 1819					
Chorilia longipes Dana, 1851	KC3089	EU920919	EU920948	EU920981	EU921052
Maja squinado (Herbst, 1788)	KAC2168	DQ079723	DQ079758	DQ079799	DQ079687
Ocypodidae Ortmann, 1894					
Macrophthalmus setosus H. Milne Edwards, 1852	AMSP67934	AY583902	AY583975	AY583994	—
Potamidae Ortmann, 1896					
Geothelphusa sp.	MLP125	DQ079715	DQ079750	DQ079790	DQ079677
Portunidae Rafinesque, 1815					
Carcinus maenas (Linnaeus, 1758)	KACcama	DQ079709	DQ079744	DQ079782	DQ079669
Macropipus puber (Linnaeus, 1758)	KACmapu	DQ079722	DQ079757	DQ079798	DQ079686
Necora puber (Linnaeus, 1767)	KAC2161	DQ079724	DQ079759	DQ079800	DQ079689
Raninidae De Haan, 1839					
Cosmonotus grayi Adams & White, 1848	KC3092	EU920918	EU920949	EU920982	EU921051
Thalassinidea Latreille, 1831					
Axiidae Huxley, 1879					
Calaxius manningi Kensley et al., 2000	NTOUA-0053	EF585447	EF585458	EF585469	—
Calocarides chani Kensley, Lin & Yu, 2000	NTOUA-00423	EF585445	EF585456	EF585467	—
Eiconaxius indicus (De Man, 1907)	NTOUA-00829	EF585449	EF585460	EF585471	—
Callianassidae Dana, 1852					
Biffarius arenosus (Poore, 1975)	BaV3	DQ079705	DQ079739	DQ079775	DQ079662
Callichirus major (Say, 1818)	KAC1864	DQ079707	DQ079741	DQ079777	DQ079664
Lepidophthalmus louisianensis (Schmitt, 1935)	KAC1852	DQ079717	DQ079751	DQ079792	DQ079678
Sergio mericeae Manning & Felder, 1995	KAC1865	DQ079733	DQ079768	DQ079811	DQ079700
Callianideidae Kossmann, 1880					
Callianidea typa H. Milne Edwards, 1837	MNHNTh	EF585448	EF585459	EF585470	—
Calocarididae Ortmann, 1891					
Calastacus crosnieri Kensley & Chan 1998	NTOUA-00212	EF585446	EF585457	EF585468	—
Paracalocaris sagamiensis Sakai, 1991	NTOUA-00142	EF585453	EF585464	EF585475	—
Ctenochelidae Manning & Felder, 1991					
Ctenocheles balssi Kishinouye, 1926	530-2-1787	EF585444	EF585455	EF585466	—
Laomediidae Borradaile, 1903					
Jaxea nocturna Nardo, 1847	N/A	AF436047	AF436006	AF435986	—
Laomedia astacina de Haan, 1841	NTOUA-00366	EF585450	EF585461	EF585472	—
Micheleidae Sakai, 1992					
Meticonaxius soelae Sakai, 1992	NTOUA-00094	EF585451	EF585462	EF585473	—
Strahlaxiidae Poore, 1994					
Neaxius acanthus (H. Milne Edwards, 1878)	NTOUA-00421	EF585452	EF585463	EF585474	—
Thalassinidae Latreille, 1831					
Thalassina anomala (Herbst, 1804)	ZRC1998-.2263	AY583896	AY583969	EF585476	—
Upogebiidae Borradaile, 1903					
Austinogebia narutensis (Sakai, 1896)	NTOUA-00416	EF585443	EF585454	EF585465	—
Upogebia affinis (Say, 1818)	N/A	AF436047	AF436007	AF435987	—

(5 PRIME), and 30–100 ng extracted DNA. The thermal cycling profile conformed to the following parameters: Initial denaturation for 2 min at 94ºC followed by 30–40 cycles of 30 sec at 94ºC, 1 min at 46–58ºC (depending on gene region), 1 min at 72ºC, and a final extension of 7 min at 72ºC. PCR products were purified using filters (PrepEase™ PCR Purification 96-well Plate Kit, USB Corporation) and sequenced with ABI BigDye® terminator mix (Applied Biosystems, Foster City, CA, USA). An Applied Biosystems 9800 Fast Thermal Cycler (Applied Biosystems, Foster City, CA, USA) was used in PCR and cycle sequencing reactions, and sequencing products were run (forward and reverse) on an ABI 3730xl DNA Analyzer 96-capillary automated sequencer.

2.4. Phylogenetic analyses

Sequences were assembled and cleaned using the computer program Sequencher 4.8 (GeneCodes, Ann Arbor, MI, USA). Sequences were aligned using MUSCLE (multiple sequence comparison by log-expectation), a computer program found to be more accurate and faster than other alignment algorithms (Edgar 2004). GBlocks v0.91b (Castresana 2000) was implemented on individual datasets (16S, 18S, 28S) to omit highly divergent and poorly aligned positions (GBlocks parameters optimized for dataset and modeled after previous studies (Porter et al. 2005): minimum number of sequences for a conserved position (16S/18S/28S) = 71/74/73; minimum number of sequences for a flanking position (16S/18S/28S) = 119/124/110, maximum number of contiguous non-conserved positions (16S/18S/28S) = 8/8/8; minimum length of a block (16S/18S/28S) = 5/5/5; allowed gap positions = half/half/half). After GBlocks pruned targeted positions, our individual 16S, 18S, and 28S datasets consisted of 298, 1546, and 970 characters, respectively. Many studies have shown an increase in resolution when multiple genes are combined in phylogenetic analyses (Ahyong & O'Meally 2004; Porter et al. 2005), and, because we were constructing a phylogeny across a broad range of taxonomic levels, we concatenated our datasets into a single alignment consisting of 3139 basepairs and 145 sequences.

The model of evolution that best fit the individual datasets (16S, 18S, 28S, H3) was determined by MODELTEST 3.7 (Posada & Crandall 1998). The Maximum Likelihood (ML) analysis was conducted using RAxML (Randomized Axelerated Maximum Likelihood) (Stamatakis et al. 2005, 2007, 2008) with computations performed on the computer cluster of the Cyberinfrastructure for Phylogenetic Research Project (CIPRES) at the San Diego Supercomputer Center. The Bayesian (BAY) analysis was conduct-

ed in MrBayes v3.0b4 (Huelsenbeck & Ronquist 2001) on the Life Sciences Computational Cluster at Brigham Young University.

Likelihood settings followed the General Time Reversible Model (GTR) with a gamma distribution and invariable sites and RAxML estimated all free parameters following a partitioned dataset. Confidence in the resulting topology was assessed using non-parametric bootstrap estimates (Felsenstein 1985) with 1000 replicates and values > 50% shown on the resulting trees. Three independent BAY analyses (each consisting of four chains) were performed using parameters selected by MODELTEST. All Markov chain Monte Carlo (MCMC) algorithms ran for 10,000,000 generations, sampling one tree every 1000 generations. To ensure that independent analyses converged on similar values, we graphically compared all likelihood parameters and scores (means and variances) using the program Tracer v1.4 (Rambaut & Drummond 2007). Observation of the likelihood (-LnL) scores in Tracer v1.4 allowed us to determine burn-ins and stationary distributions for the data. Once the values reached a plateau, a 50% majority-rule consensus tree was obtained from the remaining saved trees. Posterior probabilities (pP) for clades were compared for congruence and then combined between individual analyses with values > 0.5 displayed on the BAY phylogram.

3.   Results

In total, we included 140 16S sequences, 145 18S sequences, 144 28S sequences, and 111 H3 sequences (Tab. 1). Missing data were designated as a '?' in the alignment. The optimal models of evolution selected in MODELTEST were the General Time Reversible (GTR) model (16S, 18S, and 28S) with gamma-distributed among-site rate heterogeneity and invariant sites, and the Transversion (TVM) model (H3) with gamma-distributed among-site rate heterogeneity and invariant sites (Tab. 2). Topologies derived from the ML and BAY analyses were strongly congruent, especially within the infraordinal and family-level nodes; but because the BAY analysis showed better resolution at the deeper nodes (between infraorders) we present the BAY phylogram here (Figs. 2, 3).

In the final analysis, two taxa, *Lauridromia dehaani* Rathbun, 1923 (Brachyura) and *Enoplometopus occidentalis* (Randall, 1840) (Astacidea), were removed due to questionable positioning in the ML and BAY trees. Sequence data for both of these taxa were obtained from GenBank (Tab. 1) and were missing parts of the 28S and H3 data. In some cases, *L. dehaani* and

Tab. 2. Parameters used in BAY analysis.

Gene	Base Frequencies	Rmat	Gamma Shape Parameter	Proportion of Invariable Sites
16S	0.3653, 0.0700, 0.1679, 0.3968	1.5200, 7.8467, 1.4824, 0.6539, 12.5851	0.5982	0.2713
18S	0.2449, 0.2244, 0.2928, 0.2379	1.4140, 2.7222, 1.3404, 0.9673, 4.7274	0.5912	0.4413
28S	0.2589, 0.2222, 0.3107, 0.2082	0.9205, 2.4046, 1.2803, 0.9588, 5.2504	0.4889	0.1806
H3	0.1972, 0.2724, 0.2506, 0.2798	2.2918, 6.9776, 2.8098, 1.0917, 6.9776	1.0230	0.5640

E. occidentalis grouped within their currently accepted infraorders; in other cases, however, their positions were unresolved. All alignments and analyses were rerun after the removal of these taxa.

3.1. Higher-level relationships

Results support the monophyly of the decapod suborders Dendrobranchiata (only Penaeoidea) (bootstrap support = bs = 100%, posterior probability = pP = 1.0) and Pleocyemata (bs = 83%, pP = 0.97). Reptantia is recovered as a monophyletic clade uniting Achelata, Brachyura, Astacidea, Polychelida, Glypheidea, Anomura, and Thalassinidea (bs = 65%, pP = 0.93). The informal grouping Natantia (Stenopodidea, Caridea, and Penaeoidea) is paraphyletic with all "natant" groups positioned basally to Reptantia. There is statistical support for the following pleocyemate infraorders: Achelata (bs = 99%, pP = 1.0), Astacidea (bs = 77%, pP = 1.0), Polychelida (bs = 100%, pP = 1.0), Anomura (bs = 80%, pP = 1.0), Stenopodidea (bs = 100%, pP = 1.0), Caridea (bs = 100, pP = 1.0). The infraorder Brachyura is marginally supported with ML bootstraps (bs = 64%) and posterior probabilities (pP = 0.93). Up-tree of *Paramola japonica*, likelihood and Bayesian support significantly increase (bs = 99%, pP = 1.0). The thalassinideans are recovered as a paraphyletic assemblage consisting of two strongly supported subclades (clade I, bs = 80%, pP = 1.0; clade 2, bs = 100%, pP = 1.0). Results suggest a strong affinity between species within the dendrobranchiate superfamily Penaeoidea (bs = 100%, pP = 1.0), which is sister to the remaining decapod infraorders (Figs. 2, 3).

ML analyses did not show resolution among infraorders within the Reptantia. However, within the informal Natantia there was some evidence for an affinity between Stenopodidea and Caridea (bs = 62, best ML topology not shown). The BAY analysis did not recover this relationship Stenopodidea + Caridea, but the basal position of these groups was similar in both analyses (ML & BAY). BAY analyses showed some evidence for relationships among reptant infraorders. Clade support uniting Anomura, Polychelida, Astacidea, Brachyura, Glypheidea, and Achelata was marginally significant (pP = 0.90), and there was some evidence for affinity between the subclade Polychelida + Astacidea + Brachyura + Glypheidea (pP = 0.90) within the aforementioned group.

There is little support for the position of Glypheidea and Polychelida in relation to other "reptant" infraorders within the tree's topology. In the BAY analysis (best topology determined with -ln likelihood scores), glypheids are placed as the sister group to Achelata, and polychelids are united in a larger clade with Astacidea, Brachyura, Glypheidea, and Achelata.

## 3.2. Family-level relationships

Because many families are not sufficiently sampled, we cannot comment on their monophyly (Tab. 1). However, our findings suggest polyphyly within the families Hippolytidae (Caridea), Axiidae (Thalassinidea), Nephropidae and Cambaridae (Astacidea). More extensive taxon sampling within these families is required before we can draw definite conclusions on the monophyly of these groups, but preliminary results suggest taxonomic revisions may be needed.

4. Discussion

4.1. Infraordinal relationships

Perhaps the most debated and unresolved issue surrounding decapod evolution is rectifying infraordinal relationships, especially within the reptant lineages. Almost every combination of infraordinal relationships has been suggested, using both morphological

0.01 substitutions/site
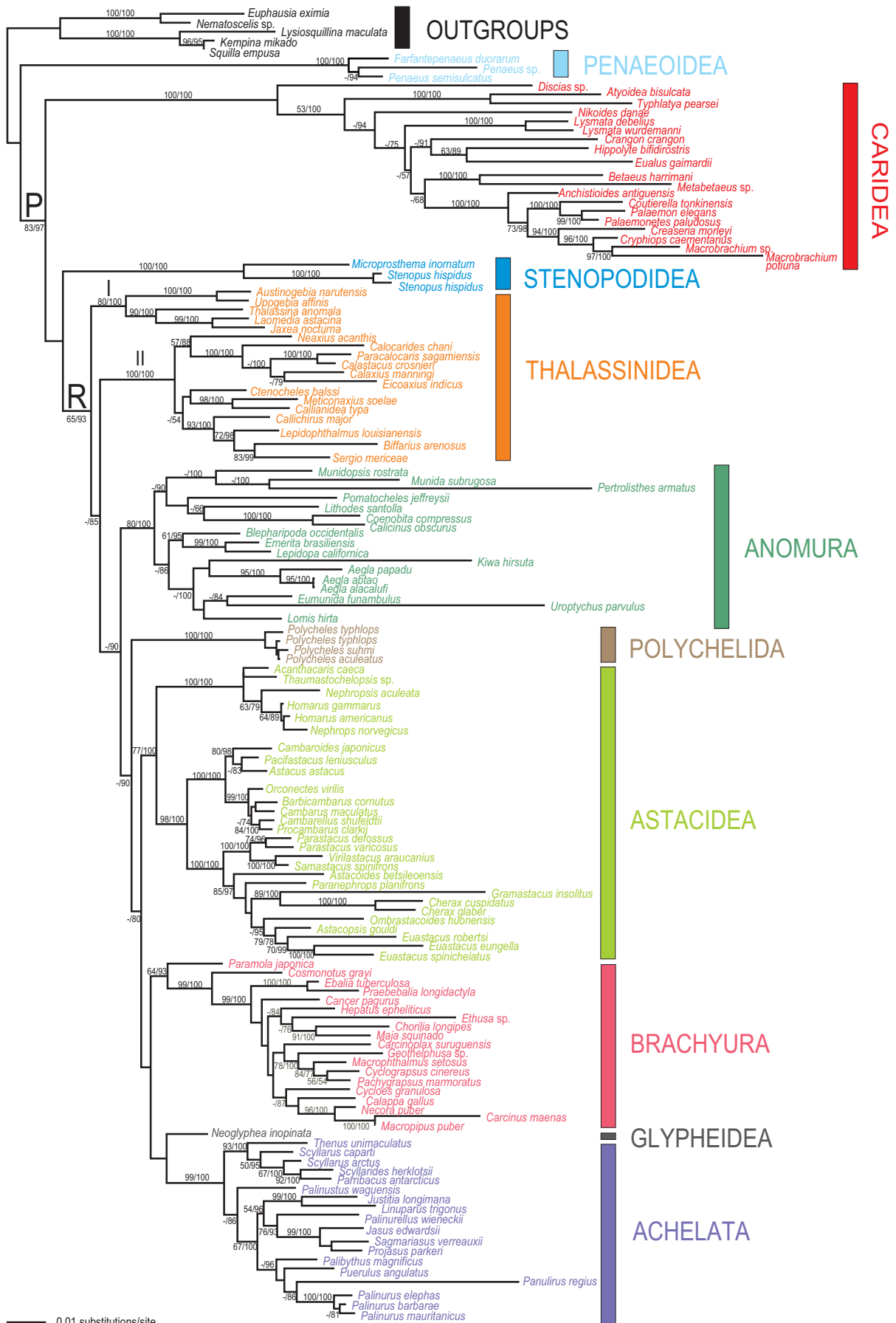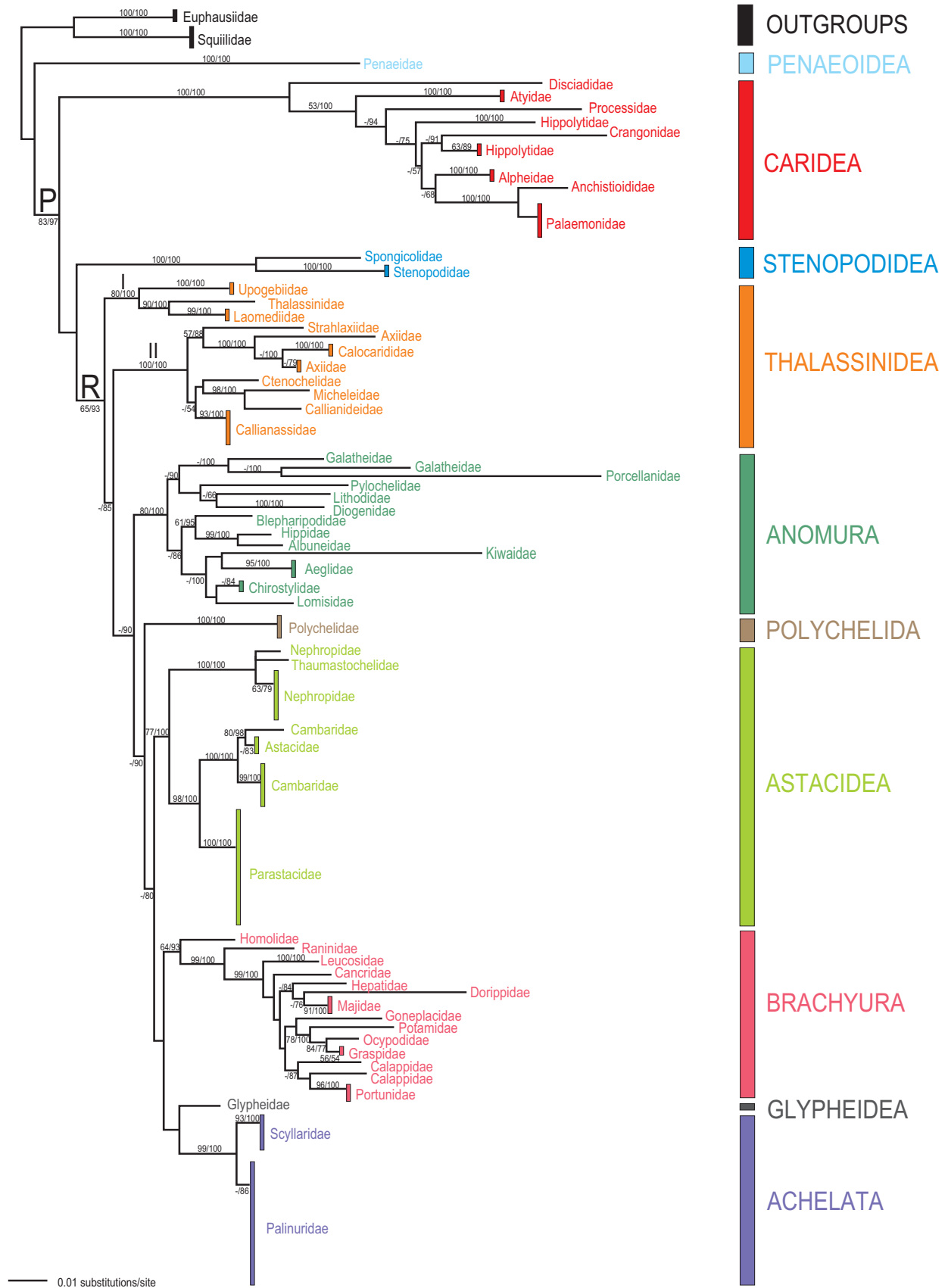
Figs. 2, 3. Species Tree (2) and family tree (3): Bayesian (BAY) phylograms for the order Decapoda (n = 131) and selected outgroup taxa (n = 5) based on a 16S (mtDNA), 18S (nDNA), 28S (nDNA) and H3 (nDNA) concatenated dataset. As compared to Fig. 2, in Fig. 3 branches are collapsed to show decapod families (n = 58). ML bootstrap values and BAY posterior probabilities are noted above or below the branches (ML/BAY), all represented as percentages (% sign omitted). Values < 50% are not shown. Vertical colored bars indicate major infraorders/superfamilies within Decapoda. I = Gebiidea; II = Axiidea; P = Pleocyemata; R = Reptantia.

and molecular data, and we are still far from reaching consensus (Fig. 1). Unfortunately, our analysis did not find any overwhelming statistical support for a single pattern of relationship among infraorders within the Reptantia. Although BAY analyses recovered marginal support for groupings among some clades (e.g., Polychelida + Astacidea + Brachyura + Glypheidea + Achelata, $pP = 0.90$), these findings need to be interpreted with caution as many studies have shown posterior probabilities to overestimate phylogenetic support (Suzuki et al. 2002; Cummings et al. 2003; Douady et al. 2003), especially on short branches. It should be noted that our findings present a hypothesis of decapod evolution that differs from those of all other recent phylogenetic studies (see Figs. 1, 2) (Crandall et al. 2000; Dixon et al. 2003; Ahyong & O'Meally 2004; Porter et al. 2005; Tsang et al. 2008b; Toon et al. 2009).

From a molecular standpoint, the lack of general agreement may stem from a shortage of phylogenetically informative markers at deeper nodes, which has resulted in conflicting tree topologies. Until recently, there have not been strong support values in any molecular analysis examining infraordinal relationships. However, Tsang et al. (2008) discovered two nuclear-protein coding genes (Nak, PEPCK) that showed promise for resolving these relationships, especially within the Reptantia. In addition to resolving higher-level relationships, the authors argued that these genes provide resolution at generic and species level phylogenies. The combination of these new genes with markers used in the present study (16S, 18S, 28S, and H3) will likely provide future insights into the evolutionary relationships within the decapod Tree of Life.

4.2. Natant lineages: Caridea, Stenopodidea, and Penaeoidea

Penaeoidea, Caridea, and Stenopodidea represent early branching lineages within the Decapoda. Insufficient taxon sampling within the penaeoids and stenopodideans does not allow us to comment on the internal relationships within these groups; however, the relationships recovered among the carideans are congruent with the only comprehensive molecular phylogeny to date (Bracken et al. 2009). The family Disciadidae, containing shallow-water tropical shrimp species, appears as the sister group to the remaining carideans, which is in conflict with previous morphological studies (Thompson 1967; Chace 1992; Holthuis 1993) and the current classification of the Caridea (Martin & Davis 2001). However, this relationship was also recovered in the molecular phylogeny of Bracken et al. (2009), which included a much more robust repre-

sentation of caridean taxa. The position of the Atyidae, an early branch in caridean evolutionary history, agrees with past suggestions and current molecular evidence (Felgenhauer & Abele 1983, 1985, 1989; Christoffersen 1990; Bracken et al. 2009). Felgenhauer & Abele (1983, 1985, 1989) suggested that the atyids are a primitive lineage on the basis of foregut morphology, which they argue is more indicative of evolutionary history than are feeding and diet. Christoffersen (1990), who nested Disciadidae and Atyidae within the superfamily Atyoidea (united by the length of the exopods of maxilliped 1 and pereopods), argued that both families contained primitive morphological characters and suggested these lineages branched early in the evolution of the Caridea.

The diverse family Hippolytidae was recovered as polyphyletic suggesting the partitioning of the group, as formally suggested in previous publications (Gurney 1942; Christoffersen 1987, 1990; Chace 1997; Bracken et al. 2009). There is strong support for an affinity between Anchistioididae and Palaemonidae, which together with six additional families (not included in analysis) constitute the superfamily Palaemonoidea. Although Palaemonidae forms a monophyletic clade, the inclusion of additional taxa may uncover polyphyletic relationships, as proposed in other analyses (Mitsuhashi et al. 2007; Bracken et al. 2009). A polyphyletic Palaemonidae is not unexpected owing to the high degree of morphological diversity within these shrimps.

4.3. Thalassinidea

The thalassinideans, or "ghost-shrimps," are a group of marine-burrowing decapods. They have a characteristic elongated body equipped with morphological adaptations which facilitate their fossorial behavior and lifestyle. Because thalassinidean burrows and bioturbating activities can have a major impact on community structure, sedimentology, and geochemical properties of benthic habitats, this group has become the focus of much research (Felder 2001; Felder & Robles 2009; Robles et al. 2009).

Over the years, there has been much debate surrounding the higher-level relationships within the Thalassinidea, and more specifically on the monophyly of the infraorder. Larval, sperm and gastric morphology suggest a paraphyletic Thalassinidea (Gurney 1938; Tudge 1995, 1997; Sakai 2005), whereas cladistic analyses based on a suite of morphological characters have recovered conflicting results supporting paraphyly (Tudge et al. 2000; Dixon et al. 2003; Ahyong & O'Meally 2004) or monophyly of the group (Poore 1994; Scholtz & Richter 1995; Schram 2001). In more recent years, molecular evi-

dence based on nuclear and mitochondrial genes has provided overwhelming evidence for the division of the infraorder (Morrison et al. 2002; Ahyong & O'Meally 2004; Tsang et al. 2008a,b; Robles et al. 2009), with two subclades strongly supported. Within recent analyses based upon a broad representation of ingroup and outgroup taxa (Robles et al. 2009), it has been proposed that the thalassinideans be separated into two infraorders, the Gebiidea and the Axiidea.

Our findings are in accord with these recent genetic results and support the paraphyly of the thalassinideans. We recovered two strongly supported subclades (I & II in Figs. 2, 3): subclade I uniting the families Upogebiidae, Thalassinidae, and Laomediidae (Gebiidea), and subclade II including Strahlaxiidae, Axiidae, Ctenochelidae, Micheleidae, Callianideidae, and Callianassidae (Axiidea). This division of the former infraorder Thalassinidea and the relationships recovered therein are directly supported by morphological evidence that includes larval, gastric, and second pereopodal characters (Gurney 1938; Saint Laurent 1979; Tudge 1995; Tudge & Cunningham 2002; Sakai 2005).

4.4. Astacidea

In our analysis, the astacideans form three strongly supported subclades, which correspond to the superfamilies Astacoidea, Parastacoidea, and Nephropoidea. Within the Astacidea, the higher-level relationships are well-understood and well-documented (Crandall et al. 2000; Rode & Babcock 2003). The freshwater crayfish are monophyletic and composed of two superfamilies, Astacoidea and Parastacoidea, each with a center of diversification within the Northern and Southern Hemisphere, respectively. The clawed lobsters (Nephropoidea) are close relatives of the freshwater crayfish, with molecular evidence securely positioning them as the sister clade to this group (Crandall et al. 2000). Although higher-level relationships within Astacidea are resolved, there is still some debate on the classification and relationships at the generic and species level (Crandall et al. 2000; Braband et al. 2006; Buhay & Crandall 2008).

The Northern Hemisphere crayfish families, Cambaridae and Astacidae, form the sister clade to the Southern Hemisphere crayfish family Parastacidae (Fig. 3). The family Cambaridae is paraphyletic, with the genus *Cambaroides* as sister to the family Astacidae (Fig. 2). The relationship between *Cambaroides* and Astacidae has been shown in past molecular analyses (Braband et al. 2006), and it is evident that a more thorough examination of the east Asian freshwater genus *Cambaroides* is needed. The position

of Thaumastochelidae is unresolved with respect to *Acanthacaris* and the other nephropids (Fig. 3). Our findings question the familial status of thaumastochelids, as do many recent molecular analyses (Tsang et al. 2008b; Tshudy et al. 2009).

4.5. Achelata

As previously discussed (see section 2.1.), we chose to recognize the infraorder Achelata because morphological and molecular evidence has recovered Palinura (comprising Achelata, Polychelida, and Glypheidea) as a polyphyletic group. The achelatan lobsters are a monophyletic group, all members of which share achelate 1st pereopods and a unique larval stage known as the phyllosoma. The infraorder traditionally contains three families: Palinuridae (spiny lobsters), Scyllaridae (slipper lobsters), and Synaxidae (coral or furry lobsters). The relationships among palinurid genera have received a lot of attention due to the economic importance of spiny lobsters in commercial fisheries (Ptacek et al. 2001; Patek & Oakley 2003; Palero et al. 2008, 2009). In contrast, few studies have focused on the scyllarids and synaxids (Palero et al. 2009). There are conflicting hypotheses of higher-level relationships among the three families based on paleontological (Förster 1973) and morphological data (Bate 1881; Holthuis 1991). Fossil data suggest a paraphyletic Palinuridae, with Scyllaridae nested within the palinurids, and morphological evidence suggests all three families to be monophyletic. Many molecular and morphological analyses have lacked sufficient sampling across the achelatan lobsters (Dixon et al. 2003; Ahyong & O'Meally 2004; Schram & Dixon 2004; Porter et al. 2005; Tsang et al. 2008b), but a recent molecular analysis based on nuclear and mitochondrial genes recognized a monophyletic Scyllaridae and polyphyletic Synaxidae (Palero et al. 2009). Palero et al. (2009) found synaxids nested within palinurids, and they proposed that only two families be recognized, namely Palinuridae and Scyllaridae. Our molecular analysis supports the finding of Palero et al. (2009) and thus supports similar familial classification, which we follow herein (Tab. 1, Fig. 3). Scyllaridae was strongly supported as a monophyletic group (Fig. 3).

4.6. Polychelida and Glypheidea

The exact position of these two infraorders is unresolved in our analysis. Polychelid and glypheid lobsters were traditionally grouped within the infraorder Palinura until molecular and morphological analyses proposed a new classification and divided the Pal-

inura into three separate infraorders, namely Achelata, Glypheidea, and Polychelida (Scholtz & Richter 1995; Dixon et al. 2003; Ahyong & O'Meally 2004).

Polychelids are unique among the reptant decapods in possessing reduced eyes (in extant species) and chelae on pereopods 1–4, sometimes 5 (Ahyong 2009). A recent cladistic analysis inferred the phylogenetic relationships within extant and fossil polychelidan lobsters (Ahyong 2009), although the position of Polychelida among reptant decapods remains unclear. Although we found support ($pP = 0.90$) for a relationship between Polychelida, Astacidea, Brachyura, Glypheidea, and Achelata, this clade is not supported by bootstrap analysis. Several analyses have suggested conflicting hypotheses on the phylogenetic position of the Polychelida (Fig. 1D,E,I,J), and our findings would suggest yet another possible classification scheme. Morphological and some molecular analyses place the polychelids as sister to the remaining reptant lineages (Scholtz & Richter 1995; Schram 2001; Dixon et al. 2003; Ahyong & O'Meally 2004), whereas recent molecular evidence suggests a sister relationship between polychelids and achelatan lobsters (Tsang et al. 2008b; Toon et al. 2009). Even in the midst of conflicting relationships, all analyses recognize Polychelida as a monophyletic clade and secure its infraordinal status among the decapods.

The glypheid lobsters were thought to be extinct until 1975, when the species *Neoglyphea inopinata* was discovered from the Philippines (Forest & Chace 1976). In the past, many researchers grouped glypheid lobsters within the infraorder Palinura because both groups possess achelate or subchelate pereopods. The discovery of *Neoglyphea inopinata* stimulated new research on the group, and subsequently the glypheid lobsters were recognized as a separate infraorder. Based on morphological cladistic analyses (Dixon et al. 2003) and molecular data (Ahyong & O'Meally 2004), Glypheidea has been suggested as the sister clade to Astacidea (recognized as Astacura) (Fig. 1). Although our analysis instead places Glypheidea as the sister group to Achelata, there is no significant support for this relationship (Figs. 2, 3).

4.7. Anomura and Brachyura

The anomurans, including hermit crabs, mole crabs, king crabs, squat lobsters and porcelain crabs, represent a broad array of body forms and functions and are among the most diverse for any group of decapods. As currently defined, Anomura contains 7 superfamilies, 17 families, and approximately 1,500 species (Ahyong et al. 2009). Although thalassinidean shrimp were once included within the anomurans, this relationship has been rejected by many phylogenetic

analyses, and today there is no dispute in their being considered separate from the anomurans (Scholtz & Richter 1995; Crandall et al. 2000; Schram 2001; Dixon et al. 2003; Ahyong & O'Meally 2004; Porter et al. 2005; Tsang et al. 2008b; Toon et al. 2009). The monophyly of Anomura is generally accepted, though the internal relationships among families, genera, and species are far from being resolved. Most hypotheses for anomuran relationships are based on morphological cladistic analyses (see Ahyong et al. 2007 for a review), and few have attempted to reconcile internal relationships using molecular data (Ahyong et al. 2009). The most recent and comprehensive molecular analyses based on mitochondrial and nuclear genes recovered support at the family level and for some superfamilies but lacked strong support at the deeper nodes (Ahyong et al. 2009). These findings are congruent with our results and suggest a polyphyletic Galatheoidea (sensu McLaughlin et al. 2007: Galatheidae + Porcellanidae + Chirostylidae, see Fig. 3). In both analyses, Chirostylidae is nested within a larger clade consisting of the families Kiwaidae, Lomisidae, and Aeglidae. Similar relationships also were found between the families Blepharipodidae, Hippidae, and Albuneidae.

The brachyurans, or true crabs, are the largest group among decapod crustaceans with almost 7,000 species (Ng et al. 2008). Our analyses support the monophyly of the Brachyura with marginal support, however values significantly increase upon removal of *Paramola japonica*. Brachyuran crabs are traditionally divided into two major groups, the Eubrachyura or "advanced crabs" and Podotremata or "primitive crabs". Podotremes are united by the presence of gonopores on the coxae of the pereopods, and they have been suggested to be monophyletic (Guinot et al. 1994), paraphyletic (Ahyong & O'Meally 2004; Ahyong et al. 2007; Scholtz & McLay 2009), or polyphyletic (Spears et al. 1992) (see Ahyong et al. 2007 for a complete review). Most recent phylogenetic studies based on molecular (Ahyong et al. 2007) and morphological data (Scholtz & McLay 2009) recover a paraphyletic Podotremata leading to a new classification for podotreme crabs (Ahyong et al. 2007). These results are congruent with our findings. We found strong evidence to suggest that Homolidae and Raninidae branched early in the evolution of the Brachyura and that Eubrachyura is a monophyletic clade. Internal relationships within the Eubrachyura (all families excluding Raninidae and Homolidae) are less stable, but we do see strong support for a clade uniting Potamidae, Ocypodidae, and Grapsidae (Fig. 3).

Numerous morphological and molecular analyses have proposed an Anomura + Brachyura clade (Meiura) (Ahyong & O'Meally 2004; Miller & Austin

2006; CARAPELLI et al. 2007; TSANG et al. 2008b), while others have proposed a different relationship (Fig. 1). Although our topology suggests an affinity between Brachyura and the Achelata + Glypheidea clade, these results must be viewed with caution as there is no statistical support for this relationship.

5.   Conclusion

Our study intended to explore the relationships among and within the infraorders and compare our findings with present hypotheses of decapod evolution. Our molecular phylogeny represents the most comprehensive sampling of Decapoda, and it is congruent with past hypotheses of monophyly within higher-level groupings, including Dendrobranchiata, Pleocyemata, and Reptantia. As expected, the Natantia, which includes the swimming lineages Caridea, Stenopodidea, and Penaeoidea, was recovered as paraphyletic. All pleocyemate infraorders and dendrobranchiate superfamilies were retained as monophyletic clades, excluding Thalassinidea. For many years, morphological and molecular studies have questioned the monophyly of thalassinideans. Our findings are in agreement with recent molecular evidence, which suggests the group is in need of major taxonomic revision.

Molecular markers are now available that can resolve deep and shallow relationships within the Decapod Tree of Life. Moving towards a resolved phylogeny requires us to build on the molecular dataset we have available. Even though it is evident that the relationships between all infraorders and many families are still under dynamic debate, the addition of taxa and compilation of new phylogenetically informative genes can lead us toward a consensus model of decapod evolution.

6.   Acknowledgments

7.   References

ABELE, L.G. & B.E. FELGENHAUER 1986. Phylogenetic and phenetic relationships among the lower Decapoda. – Journal of Crustacean Biology **6**: 385–400.

AHYONG, S.T. & D. O'MEALLY 2004. Phylogeny of the Decapoda Reptantia: Resolution using three molecular loci and morphology. – The Raffles Bulletin of Zoology **52**: 673–693.

AHYONG, S.T. 2009. The polychelidan lobsters: phylogeny and systematics (Polychelida: Polychelidae). Pp. 360–390 *in*: J.W. MARTIN, D.L. FELDER & K.A. CRANDALL (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

AHYONG, S.T., J.C.Y. LAI, D. SHARKEY, D.J. COLGAN & P.K.L. NG 2007. Phylogenetics of the brachyuran crabs (Crustacea: Decapoda): The status of Podotremata based on small subunit nuclear ribosomal RNA. – Molecular Phylogenetics and Evolution **45**: 576–586.

AHYONG, S.T., K.E. SCHNABEL & E.W. MAAS 2009. Anomura phylogeny: New insights from molecular data. Pp. 391–408 *in*: J.W. MARTIN, D.L. FELDER & K.A. CRANDALL (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

BATE, C.S. 1881. On *Synaxes*, a new genus of Crustacea. – Annals and Magazine of Natural History **5**: 220–228.

BOAS, F.E.V. 1880. Studier over decapodernes slaegtskabsforhold. – Dansk Videnskabernes Seksjeab, Copenhagen, Skrifter, Naturvidenskabelig og matematisek Afdeling **1**: 26–210.

BRABAND, A., T. KAWAI & G. SCHOLTZ 2006. The phylogenetic position of the East Asian freshwater crayfish Cambaroides within the Northern Hemisphere Astacoidea (Crustacea, Decapoda, Astacida) based on molecular data. – Journal of Zoological Systematics and Evolutionary Research **44**: 17–24.

BRACKEN, H.D., S. DE GRAVE & D.L. FELDER 2009. Phylogeny of the infraorder Caridea based on nuclear and mitochondrial genes (Crustacea: Decapoda). Pp. 274–300 *in*: J.W. MARTIN, D.L. FELDER & K.A. CRANDALL (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton.

BUHAY, J.E. & K.A. CRANDALL 2008. Taxonomic revision of cave crayfishes in the genus *Orconectes*, subgenus *Orconectes* (Decapoda: Cambaridae) along the Cumberland Plateau, including a description of a new species, *Orconectes barri*. – Journal of Crustacean Biology **28**: 57–67.

BURKENROAD, M.D. 1963. The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record. – Tulane Studies in Geology **2**: 1–18.

BURKENROAD, M.D. 1981. The higher taxonomy and evolution of Decapoda (Crustacea). – Transactions of the San Diego Society of Natural History **19**: 251–268.

CARAPELLI, A., P. LIO, F. NARDI, E. VAN DER WATH & F. FRATI 2007. Phylogenetic analysis of mitochondrial protein coding genes confirms the reciprocal paraphyly of Hexapoda and Crustacea. – BMC Evolutionary Biology **7** (Suppl. 2): S8.

CASTRESANA, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – Molecular Biology and Evolution **17**: 540–552.

Chace, F.A. 1992. On the classification of the Caridea (Decapoda). – Crustaceana 63: 70–80.

Chace, F.A. 1997. The caridean shrimps (Crustacea: Decapoda) of the Albatross Philippine Expedition, 1907–1910, Part 7: Families Atyidae, Eugonatonotidae, Rhynchocinetidae, Bathypalaemonellidae, Processidae, and Hippolytidae. – Smithsonian Contributions to Zoology 587: 1–106.

Christoffersen, M.L. 1987. Phylogenetic relationships of hippolytid genera, with an assignment of new families for the Crangonoidea and Alpheoidea (Crustacea, Decapoda, Caridea). – Cladistics 3: 348–362.

Christoffersen, M.L. 1988. Phylogenetic systematics of the Eucarida (Crustacea, Malacostraca). – Revista Brasiliera de Zoologia 5: 325–351.

Christoffersen, M.L. 1990. A new superfamily classification of the Caridea (Crustacea: Pleocyemata) based on phylogenetic pattern. – Zeitschrift für Zoologische Systematik und Evolutionsforschung 28: 94–106.

Colgan, D.J., A. McLauchlan, G.D.F. Wilson, S.P. Livingston, G.D. Edgecombe, J. Macaranas, G. Cassis & M.R. Gray 1998. Histone 3 and U2 snRNA DNA sequences and arthropod molecular evolution. – Australian Journal of Zoology 46: 419–437.

Crandall, K.A., J.D. Harris & J.W. Fetzner 2000. The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. – Proceedings of the Royal Society of London B 267: 1679–1686.

Crandall, K.A. & J.F. Fitzpatrick 1996. Crayfish molecular systematics: Using a combination of procedures to estimate phylogeny. – Systematic Biology 45: 1–26.

Cummings, M.P., S.A. Handley, D.S. Myers, D.L. Reed, A. Rokas & K. Winka 2003. Comparing bootstrap and posterior probability values in the four-taxon case. – Systematic Biology 52: 477–487.

Dixon, C.J., S. Ahyong & F.R. Schram 2003. A new hypothesis of decapod phylogeny. – Crustaceana 76: 935–975.

Douady, C.J., F. Delsuc, Y. Boucher, W.F. Doolittle & E.J.P. Douzery 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. – Molecular Biology and Evolution 20: 248–254.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. – Nucleic Acids Research 32: 1792–1797.

Felder, D.L. 2001. Diversity and ecological significance of deep-burrowing macrocrustaceans in coastal tropical waters of the americas (Decapoda: Thalassinidea). – Interciencia 26: 440–449.

Felder, D.L. & R. Robles 2009. Molecular phylogeny of the family Callianassidae based on preliminary analyses of two mitochondrial genes. Pp. 319–334 in: J.W. Martin, D.L. Felder & K.A. Crandall (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

Felgenhauer, B.E. & L.G. Abele 1983. Phylogenetic relationships among shrimp-like decapods. Pp. 291–311 in: F.R. Schram (ed.), Crustacean Phylogeny. – A.A. Balkema, Rotterdam.

Felgenhauer, B.E. & L.G. Abele 1985. Feeding structures of 2 atyid shrimps, with comments on caridean phylogeny. – Journal of Crustacean Biology 5: 397–419.

Felgenhauer, B.E. & L.G. Abele 1989. Evolution of the foregut in the lower Decapoda. Pp. 205–219 in: B.E. Felgenhauer, L. Watling & A.B. Thistle (eds.), Functional Morphology of Feeding and Grooming in Crustacea. – A.A. Balkema, Rotterdam.

Felsenstein, J. 1985. Confidence-limits on phylogenies with a molecular clock. – Systematic Zoology 34: 152–161.

Forest, J. & F.A. Chace 1976. Neoglyphea inopinata: a crustacean "living fossil" from the Phillippines. – Science 192: 884.

Förster, R. 1973. Untersuchungen an oberjurassischen Palinuridae. – Mitteilungen der Bayerischen Staatssammlung für Palaeontologie und Historische Geologie 13: 31–46.

Giribet, G., S. Carranza, J. Baguna, M. Riutort & C. Ribera 1996. First molecular evidence for the existence of a Tardigrada plus Arthropoda clade. – Molecular Biology and Evolution 13: 76–84.

Guinot, D., B.G.M. Jamieson & B.R. Deforges 1994. Relationship of Homolidae and Dromiidae – Evidence from spermatozoal ultrastructure (Crustacea, Decapoda). – Acta Zoologica 75: 255–267.

Gurney, R. 1938. The Larvae of the Decapod Crustacea: Palaemonidae and Alpheidae. Great Barrier Reef Expedition 1928–29, Scientific Reports 6: 1–60.

Gurney, R. 1942. Larvae of Decapod Crustacea. – Ray Society, London. 306 pp.

Holthuis, L.B. 1991. FAO species catalog. Vol. 13. Marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date. – Food and Agriculture Organization of the United Nations, Rome. 292 pp.

Holthuis, L.B. 1993. The Recent Genera of the Caridean and Stenopodidean Shrimps (Crustacea, Decapoda): with an Appendix on the Order Amphionidacea. – Nationaal Natuurhistorisch Museum, Leiden. 328 pp.

Huelsenbeck, J.P. & F. Ronquist 2001. MRBAYES: Bayesian inference of phylogeny. – Biometrics 17: 754–755.

Mantelatto, F.L.M., R. Robles & D.L. Felder 2007. Molecular phylogeny of the western Atlantic species of the genus Portunus (Crustacea, Brachyura, Portunidae). – Zoological Journal of the Linnean Society 150: 211–220.

Mantelatto, F.L.M., R. Robles, R. Biagi & D.L. Felder 2006. Molecular analysis of the taxonomic and distributional status for the hermit crab genera Loxopagurus Forest, 1964 and Isocheles Stimpson, 1858 (Decapoda, Anomura, Diogenidae). – Zoosystema 28: 495–506.

Martin, J.W. & G.E. Davis 2001. An updated classification of the Recent Crustacea. – Natural History Museum of Los Angeles County, Science Series 39: 1–124.

McLaughlin, P.A., R. Lemaitre & U. Sorhannus 2007. Hermit crab phylogeny: A reappraisal and its "fallout". – Journal of Crustacean Biology 27: 97–115.

Miller, A.D. & C.M. Austin 2006. The complete mitochondrial genome of the mantid shrimp Harpiosquilla harpax, and a phylogenetic investigation of the Decapoda using mitochondrial sequences. – Molecular Phylogenetics and Evolution 38: 565–574.

Mitsuhashi, M., Y.W. Sin, H.C. Lei, T.-Y. Chan & K.H. Chu 2007. Systematic status of the caridean families Gna-

thophyllidae Dana and Hymenoceridae Ortmann (Crustacea: Decapoda): a preliminary examination based on nuclear rDNA sequences. – Invertebrate Systematics **21**: 613–622.

MORRISON, C.L., A.W. HARVEY, S. LAVERY, K. TIEU, Y. HUANG & C.W. CUNNINGHAM 2002. Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. – Proceedings of the Royal Society of London B **269**: 345–350.

NG, P.K.L., D. GUINOT & P.J. DAVIE 2008. Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. – Raffles Bulletin of Zoology **17**: 1–286.

PALERO, F., P. ABELLO, E. MACPHERSON, M. GRISTINA & M. PASCUAL 2008. Phylogeography of the European spiny lobster (*Palinurus elephas*): Influence of current oceanographical features and historical processes. – Molecular Phylogenetics and Evolution **48**: 708–717.

PALERO, F., K.A. CRANDALL, P. ABELLÓ, E. MACPHERSON & M. PASCUAL 2009. Phylogenetic relationship between spiny, slipper and coral lobsters (Crustacea, Decapoda, Achelata). – Molecular Phylogenetics and Evolution **50**: 152–162.

PATEK, S.N. & T.H. OAKLEY 2003. Comparative tests of evolutionary trade-offs in a palinurid lobster acoustic system. – Evolution **57**: 2082–2100.

POORE, G.C.B. 1994. A phylogeny of the families of Thalassinidea (Crustacea: Decapoda) with keys to families and genera. – Memoirs of the Museum of Victoria **54**: 79–120.

PORTER, M.L., M. PEREZ-LOSADA & K.A. CRANDALL 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. – Molecular Phylogenetics and Evolution **37**: 355–369.

POSADA, D. & K.A. CRANDALL 1998. MODELTEST: Testing the model of DNA substitution. – Bioinformatics **14**: 817–818.

PTACEK, M.B., S.K. SARVER, M.J. CHILDRESS & W.F. HERRNKIND 2001. Molecular phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). – Marine and Freshwater Research **52**: 1037–1047.

RAMBAUT, A. & A.J. DRUMMOND 2007. Tracer v1.4. – Available from http://beast.bio.ed.ac.uk/Tracer.

ROBLES, R., C.D. SCHUBART, J.E. CONDE, C. CARMONA-SUAREZ, F. ALVAREZ, J.L. VILLALOBOS & D.L. FELDER 2007. Molecular phylogeny of the American *Callinectes* Stimpson, 1860 (Brachyura: Portunidae), based on two partial mitochondrial genes. – Marine Biology **150**: 1265–1274.

ROBLES, R., C.C. TUDGE, P.C. DWORSCHAK, G.C.B. POORE & D.L. FELDER 2009. Molecular phylogeny of the Thalassinidea based on nuclear and mitochondrial genes. Pp. 301–318 *in*: J.W. MARTIN, D.L. FELDER & K.A. CRANDALL (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

RODE, A.L. & L.E. BABCOCK 2003. Phylogeny of fossil and extant freshwater crayfish and some closely related nephropid lobsters. – Journal of Crustacean Biology **23**: 418–435.

SAINT LAURENT, M.d. 1979. Sur la classification et la phylogénie des Thalassinides: définitions de la superfamille des Axioidea, de la sous-famille des Thomassiniinae

et de deux genres nouveaux (Crustacea Decapoda). – Comptes Rendus Hebdomadaires de Séances de l'Académie des Sciences, Paris **288**: 1395–1397.

SAKAI, K. 2005. The diphyletic nature of the infraorder Thalassinidea (Decapoda: Pleocyemata) as dervided from the morphology of the gastric mill. – Crustaceana **77**: 1117–1129.

SCHOLTZ, G. & C.L. MCLAY 2009. Is the Brachyura Podotremata a monophyletic group? Pp. 409–427 *in*: J.W. MARTIN, D.L. FELDER & K.A. CRANDALL (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

SCHOLTZ, G. & S. RICHTER 1995. Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). – Zoological Journal of the Linnean Society **113**: 289–328.

SCHRAM, F.R. 2001. Phylogeny of decapods: moving towards a consensus. – Hydrobiologia **449**: 1–20.

SCHRAM, F.R. & C.J. DIXON 2004. Decapod phylogeny: addition of fossil evidence to a robust morphological cladistic data set. – Bulletin of the Mitzunami Fossil Museum **31**: 1–19.

SCHUBART, C.D., J.E. NEIGEL & D.L. FELDER 2000. Use of the mitochondrial16S rRNA gene for phylogenetic and population studies of Crustacea. – Crustacean Issues **12**: 817–830.

SPEARS, T., L.G. ABELE & M.A. APPLEGATE 1994. Phylogenetic study of cirripedes and selected relatives (Thecostraca) based on 18S rDNA sequence analysis. – Journal of Crustacean Biology **14**: 641–656.

SPEARS, T., L.G. ABELE & W. KIM 1992. The monophyly of brachyuran crabs: A phylogenetic study based on 18S rRNA. – Systematic Biology **41**: 446–461.

STAMATAKIS, A., F. BLAGOJEVIC, D.S. NIKOLOPOULOS & C.D. ANTONOPOULOS 2007. Exploring new search algorithms and hardware for phylogenetics: RAxML meets the IBM cell. – Journal of Vlsi Signal Processing Systems for Signal Image and Video Technology **48**: 271–286.

STAMATAKIS, A., P. HOOVER & J. ROUGEMONT 2008. A rapid bootstrap algorithm for the RAxML web servers. – Systematic Biology **57**: 758–771.

STAMATAKIS, A., T. LUDWIG & H. MEIER 2005. RAxML-III: A fast program for maximum likelihood-based inference of large phylogenetic trees. – Bioinformatics **21**: 456–463.

STILLMAN, J.H. & C.A. REEB 2001. Molecular phylogeny of eastern Pacific porcelain crabs, genera *Petrolisthes* and *Pachycheles*, based on the mtDNA 16S rDNA sequence: Phylogeographic and systematic implications. – Molecular Phylogenetics and Evolution **19**: 236–245.

SUZUKI, Y., G.V. GLAZKO & M. NEI 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. – Proceeding of the National Academy of Sciences **99**: 16138–16143.

THOMPSON, J.R. 1967. Comments on phylogeny of section Caridea (Decapoda: Natantia) and the phylogenetic importance of the Oplophoroidea. – Proceedings of Symposium on Crustacea, Part 1: 314–326.

TOON, A., M. FINLEY, J. STAPLES & K.A. CRANDALL 2009. Decapod phylogenetics and molecular evolution. Pp. 348–359 *in*: J.W. MARTIN, D.L. FELDER & K.A. CRANDALL (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

Tsang, L.M., F.J. Lin, K.H. Chu & T.Y. Chan 2008a. Phylogeny of Thalassinidea (Crustacea, Decapoda) inferred from three rDNA sequences: implications for morphological evolution and superfamily classification. – Journal of Zoological Systematics and Evolutionary Research **46**: 216–223.

Tsang, L.M., K.Y. Ma, S.T. Ahyong, T.Y. Chan & K.H. Chu 2008b. Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. – Molecular Phylogenetics and Evolution **48**: 359–368.

Tshudy, D., R. Robles, T.Y. Chan, K.C. Ho, K.H. Chu, S.T. Ahyong & D.L. Felder 2009. Phylogeny of the marine clawed lobster families Nephropidae Dana, 1852, and Thaumastochelidae Bate, 1888, based on mitochondrial and nuclear genes. Pp. 348–359 *in*: J.W. Martin, D.L. Felder & K.A. Crandall (eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). – CRC Press, Bocan Raton, FL.

Tudge, C. 1995. Ultrastructure and phylogeny of the spermatozoa of the infraorders Thalassinidea and Anomura (Decapoda, Crustacea). Pp. 251–263 *in*: B.G.M. Jamieson, J. Ausio & J.-L. Justin (eds.), Advances in Spermatozoal Phylogeny and Taxonomy. – Paris Ed. du Muséum, Paris.

Tudge, C.C. 1997. Phylogeny of the Anomura (Decapoda, Crustacea): Spermatozoa and spermatophore morphological evidence. – Contributions to Zoology **67**: 125–141.

Tudge, C.C. & C.W. Cunningham 2002. Molecular phylogeny of the mud lobsters and mud shrimps (Crustacea: Decapoda: Thalassinidea) using nuclear 18S rDNA and mitochondrial 16S rDNA. – Invertebrate Systematics **16**: 839–847.

Tudge, C.C., G.C.B. Poore & R. Lemaitre 2000. Preliminary phylogenetic analysis of generic relationships within the Callianassidae and Ctenochelidae (Decapoda: Thalassinidea: Callianassoidea). – Journal of Crustacean Biology **20**: 129–149.

Whiting, M.F. 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. – Zoologica Scripta **31**: 93–104.

Whiting, M.F., J.C. Carpenter, Q.D. Wheeler & W.C. Wheeler 1997. The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. – Systematic Biology **46**: 1–68.