Decapod Crustacean Phylogenetics

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Anomuran Phylogeny: New Insights from Molecular Data

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ABSTRACT

High-level classifications of Anomura typically recognize three major clades: Galatheoidea (squat lobsters and porcelain crabs), Paguroidea (hermit and king crabs), and Hippoidea (mole crabs). The general stability of this classification, however, has masked the vigorous debate over internal relationships. Phylogenetic relationships of the Anomura are analyzed based on sequences from three molecular loci (mitochondrial 16S; nuclear 18S and 28S), with multiple exemplars representing 16 of 17 extant families. The dataset assembled is the largest analyzed to date for Anomura. Analyses under maximum parsimony and Bayesian inference recognize a basal position for Hippoidea, corroborating several recent studies, but point to significant polyphyly in the two largest superfamilies, Galatheoidea and Paguroidea. Three independent carcinization events are identified (in Lithodidae, Porcellanidae, and Lomisidae). The polyphyletic origin of asymmetrical hermit crabs is a radical departure from previous studies and suggests independent derivations of asymmetry in three separate clades: Paguridae, Coenobitidae + Diogenidae, and Parapaguridae. Such a scenario may seem unlikely owing to the complex characters involved, but if carcinization has multiple, independent origins, then adaptation to dextral shell habitation may also be plausible. Polyphyly of Galatheoidea, however, while unexpected, is morphologically tenable—characters traditionally used to unify Galatheoidea are plesiomorphies. Chirostylid squat lobsters are more closely related to an assemblage including aegloids, lomisoids, and parapagurids than to other galatheoids. Galatheidae may be paraphyletic on the basis of an internally nested Porcellanidae, and a similar situation may obtain for Chirostylidae with respect to Kiwaidae. Present topologies are not sufficiently robust to justify significant changes to the classification, but they point to fruitful lines for further research.

1 INTRODUCTION

Few major decapod groups have had as unstable a taxonomic history as the Anomura. Historically, the composition of Anomura has been significantly fluid, with inclusion or exclusion of the major groups such as the thalassinidean shrimps and the dromiacean crabs (reviewed by Martin & Davis 2001; McLaughlin et al. 2007). Even the name has not been universally accepted, with some authors favouring Anomala over Anomura (see McLaughlin & Holthuis 1985). Most classifications recognize three major anomuran groups: Galatheoidea (squat lobsters and porcelain crabs), Paguroidea (hermit and king crabs), and Hippoidea (mole crabs). The general anomuran classification has been relatively stable for the last two to three decades, but this stability has masked the vigorous and ongoing debate over their internal relationships.

Nevertheless, advances have been made. The monophyly of Anomura is now well established. The relationship between thalassinideans and anomurans has long been ambiguous, leading workers to variously recognize independent status for each group or a single, expanded Anomura (e.g., Henderson 1888; Borradaile 1907; Balss 1957; Burkenroad 1963, 1981; Glaessner 1969;

McLaughlin 1983b). McLaughlin & Holthuis (1985) excluded thalassinideans from Anomura, and this has been corroborated by numerous phylogenetic analyses (e.g., Martin & Abele 1986; Poore 1994; Scholtz & Richter 1995; Ahyong & O'Meally 2004; Tsang et al. 2008). The dromiacean crabs, which were variously regarded as anomuran or brachyuran based largely on plesiomorphic larval features, are confirmed as Brachyura (the 'true' crabs) (see Spears et al 1992; Ahyong et al. 2007). Moreover, the sister group to Anomura is now widely accepted as Brachyura, the two clades constituting Meiura (Scholtz & Richter 1995; Schram 2001; Dixon et al. 2003; Ahyong & O'Meally 2004; Tsang et al. 2008). The ingroup for analysis is thus well circumscribed in terms of composition and monophyly.

Anomura presently includes 7 superfamilies, 17 families, almost 200 genera, and about 1500 species. Although less speciose than its sister clade by more than one-quarter, recovering the pattern of anomuran evolution is no less challenging. Anomura presents a morphological array that spans the generalized squat lobsters, symmetrical and asymmetrical hermit crabs, the brachyuran-like king and porcelain crabs, and fossorial mole crabs. Overlying this diversity is the phenomenon of carcinization (Borradaile 1916), the evolution of a crab-like form, which has occurred independently in multiple anomuran lineages. Anomurans may thus prove to be a particularly fruitful group for investigating evolution of form. Were one so inclined, the meiuran morphospace might even be viewed as an evolutionary 'testing ground' for different ground-plans, out of which the Brachyura was singularly most successful (at least numerically) and most effectively carcinized. Consequently, although highly diverse, brachyurans still exhibit a greater degree of morphological uniformity than does Anomura. Anomurans, on the other hand, emerge with a much wider array of forms, exhibiting considerably greater morphological disparity than the 'true' crabs. Discovering the connections between these morphologically disparate clades, however, presents significant challenges to phylogenetic reconstruction, not least because their conditions of existence presumably exert considerable influence on the expression of form.

The advent of cladistic analysis has seen a steady rise in efforts to understand anomuran evolution and interrelationships (Fig. 1). In addition to the increasing application of cladistic methods, mostly based on somatic morphology, new sources of data have become increasingly accessible, the most significant being DNA sequences. Most phylogenetic studies of anomurans are based on morphology, most recently McLaughlin et al. (2007); few have explored molecular data to any great extent. Thus, to reconstruct phylogenetic interrelationships of the Anomura, we assembled existing and newly generated sequence data from three molecular loci (mitochondrial 16S; nuclear 18S and 28S) encompassing 16 of 17 recognized anomuran families in the largest anomuran dataset to date.

2   MATERIALS AND METHODS

2.1   *Taxon sampling*

Representatives of all anomuran families, *sensu* McLaughlin et al. (2007) (except Pylojacquesidae), were included as terminals, with emphasis on the Galatheoidea (Table 1). Representatives of all three galatheid subfamilies were included, representing 11 of 34 recognized genera. Porcellanidae was represented by three exemplars and Chirostylidae was represented by five of six recognized genera. Tissue samples were derived from specimens in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN); National Institute of Water and Atmospheric Research, Wellington, New Zealand (NIWA); and National Taiwan Ocean University, Keelung, Taiwan (NTOU). The 28S sequence of *Shinkaia* was amplified from genomic DNA generously provided by K. H. Chu (Chinese University of Hong Kong), who also shared unpublished 16S and 18S *Shinkaia* sequences. Brachyura is the sister group to Anomura (Scholtz & Richter 1995; Ahyong & O'Meally 2004;
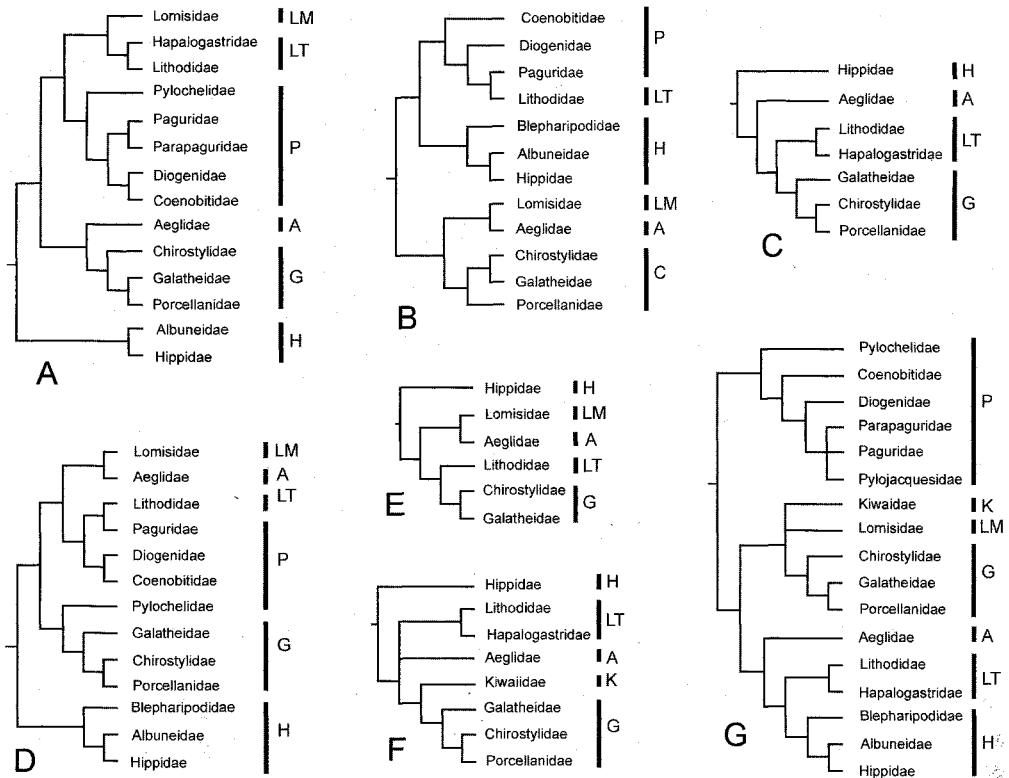
Figure 1. Selected hypotheses of anomuran relationships. (A) based on Martin & Abele (1986); (B) based on Morrison et al. (2002); (C) based on Pérez-Losada et al. (2002); (D) based on Ahyong & O'Meally (2004); (E) based on Porter et al. (2005); (F) based on Macpherson et al. (2005); (G) based on McLaughlin et al. (2007). Superfamilies as recognized by McLaughlin et al. (2007) abbreviated as follows: Aegloidea – A; Kiwaoidea – K; Galatheoidea – G; Hippoidea – H; Lithodoidea – LT; Lomisoidea – LM; Paguroidea – P.

Tsang et al. 2008), so the analysis was rooted to two brachyuran exemplars, *Lauridromia dehaani* and *Paromola japonica*.

2.2   *Molecular data*

Two nuclear ribosomal genes (18S rRNA and the D1 region of 28S rRNA) and one mitochondrial ribosomal gene (16S rRNA) were selected for their utility in resolving phylogenetic history at different taxonomic levels (Crandall et al. 2000; Ahyong & O'Meally 2004). We collected new sequence data for 19 species, resulting in 53 new sequences (see Table 1). Other sequences were available in GenBank. For the *Pagurus* terminal, 16S and 28S sequences were derived from *P. bernhardus* and the 18S sequence from *P. longicarpus*.

Table 1. Classification of terminal taxa with GenBank accession numbers for gene sequences. New sequences are indicated (*). *Shinkaia* 16S and 18S sequence provided by K. H. Chu (KHC, Chinese University of Hong Kong). For convenience, the high-level classification follows McLaughlin et al. (2007). Location of voucher specimens for new sequences: MNHN (Muséum National d'Histoire Naturelle, Paris), NIWA (National Institute of Water and Atmospheric Research, Wellington, New Zealand), NTOU (National Taiwan Ocean University, Keelung, Taiwan).

	16S	18S	28S	Voucher
ANOMURA				
AEGLOIDEA				
AEGLIDAE				
Aegla uruguyana Schmitt, 1942 (*Aegla 1*)	AF436051	AF436012	AF435992	
Aegla violacea Bond-Buckup & Buckup, 1994 (*Aegla 2*)	AY595880	AY595799	AY596051	
HIPPOIDEA				
ALBUNEIDAE				
Lepidopa californica Efford, 1971	AF436054	AF436015	AF435996	
BLEPHARIPODIDAE				
Blepharipoda occidentalis Randall, 1840	AF436053	AF436014	AF435994	
HIPPIDAE				
Emerita emeritus (Linnaeus, 1767)	AY583898	AY583971	AY583990	
KIWAOIDEA				
KIWAIDAE				
Kiwa hirsuta Macpherson, Jones & Segonzac, 2005	*EU831284	DQ219316	*EU831286	MNHN
PAGUROIDEA				
COENOBITIDAE				
Coenobita compressus H. Milne Edwards, 1837	AF436059	AF436023	AF435999	
DIOGENIDAE				
Calcinus obscurus Stimpson, 1859	AF436058	AF436022	AF435998	
Clibanarius albidigitatus Nobili, 1901	AF425323	AF438751	AF425362	
Isocheles pilosus (Holmes, 1900)	AF436057	AF436021	–	
PAGURIDAE				
Bythiopagurus macroculus McLaughlin, 2003	*EU821532	*EU821548	*EU821565	NIWA
Discorsopagurus schmitti (Stevens, 1925)	AF436055	AF436017	–	
Pagurus bernhardus (Linnaeus, 1758)	AF425335	–	AF425354	
Pagurus longicarpus Say, 1817	–	AF436018	–	
PARAPAGURIDAE				
Parapagurus latimanus Henderson, 1888	*EU821534	*EU821550	*EU821567	NIWA
Sympagurus dimorphus (Studer, 1883)	*EU821533	*EU821549	*EU821566	NIWA
PYLOCHELIDAE				
Pylocheles macrops Forest, 1987	AY583897	AY583970	AY583989	
Trizocheles spinosus (Henderson, 1888)	*EU821535	*EU821551	*EU821568	NIWA
LITHODOIDEA				
LITHODIDAE				
Lithodes santolla (Molina, 1782)	AF595927	AF439385	AF596100	
HAPALOGASTRIDAE				
Oedignathus inermis (Stimpson, 1860)	AF425334	Z104062	AF425353	

Table 1. continued.

	16S	18S	28S	Voucher
LOMISOIDEA				
LOMISIDAE				
Lomis hirta (Lamarck, 1818)	AF436052	AF436013	AF435993	
GALATHEOIDEA				
CHIROSTYLIDAE				
Chirostylus novaecaledoniae Baba, 1991	*EU821539	*EU821555	*EU821572	MNHN
Eumunida sternomaculata Saint Laurent & Poupin, 1996	AY351063	AF436011	AF435991	
Gastroptychus novaezelandiae Baba, 1974	*EU821538	*EU821554	*EU821571	NIWA
Pseudomunida fragilis Haig, 1979	*EU821536	*EU821552	*EU821569	MNHN
Uroptychus nitidus (A. Milne-Edwards, 1880)(*Uroptychus 1*)	AY595925	AF439387	AY596096	
Uroptychus scambus Benedict, 1902 (*Uroptychus 2*)	*EU831282	*EU821553	*EU831283	NIWA
GALATHEIDAE				
Galatheinae				
Agononida longipes (A. Milne-Edwards, 1880) (*Agononida 1*)	–	AF439381	–	
Agononida procera Ahyong & Poore, 2004 (*Agononida 2*)	*EU821540	*EU821556	*EU821573	NIWA
Allogalathea elegans (Adams & White, 1848)	*EU821543	*EU821560	*EU821577	MNHN
Cervimunida johni (Porter, 1903)	*EU821546	*EU821563	*EU821580	NIWA
Galathea sp.	*EU821544	*EU821561	*EU821578	NIWA
Leiogalathea laevirostris (Balss, 1913)	*EU821541	*EU821557	*EU821574	NIWA
Munida quadrispina Benedict, 1902 (*Munida 1*)	AF436050	AF436010	AF435990	
Munida gregaria (Fabricius, 1793) (*Munida 2*)	AY050075	AF439382	AY596099	
Pleuroncodes monodon (H. Milne Edwards, 1837)	*EU821545	*EU821562	*EU821579	NIWA
Sadayoshia sp.	*EU821547	*EU821564	*EU821571	MNHN
Munidopsinae				
Galacantha rostrata (A. Milne-Edwards, 1880)	–	*EU821559	*EU821576	NIWA
Munidopsis bairdii (Smith, 1884)	*EU821542	*EU821558	*EU821575	NIWA
Shinkaiinae				
Shinkaia crosnieri Baba & Williams, 1998	KHC	KHC	*EU831285	NTOU
PORCELLANIDAE				
Pachycheles rudis Stimpson, 1859	AF260598	AF436048	AF435988	
Petrolisthes armatus (Gibbes, 1850)	AF436049	AF436009	AF435989	
Porcellanella triloba White, 1851	*EU834069	–	–	

2.3   *DNA extraction and analysis*

Genomic DNA was either directly extracted from fresh or ethanol-fixed tissue samples that were soaked 24 hours in a buffer containing 500 mM Tris-HCL (pH 9.0), 20mM EDTA, and 10 mM NaCl. Extraction followed the standard protocol of the QIAGEN DNeasy Blood & Tissue Kit and subsequent quantification of DNA concentration using PicoGreen TM (Molecular Probes Inc., USA). For problematic taxa, a linear acrylamide precipitation was used overnight to increase concentration of DNA. Sequences of two nuclear (the nearly complete sequence of 18S and the 28S D1 expansion region) and one mitochondrial (16S) ribosomal RNA genes were obtained. Primers used are indicated in Table 2. Polymerase chain reactions (PCR) were conducted in 25-μL volumes with 1–5 μL of genomic DNA and using Invitrogen Platinum PCR SuperMix containing 22 mM Tris-HCL, 55 mM KCl, 1.65 mM $MgCl_2$, and 220 μM dNTP. Conditions for 18S and 28S amplification were an initial denaturation at 94°C for two minutes, then 30 cycles of 94°C for one minute, annealing for 1 minute at 50°C, extension at 72°C for two minutes, and a final extension at 72°C for seven minutes. Conditions for 16S amplification were an initial denaturation at 94°C for 5 minutes followed by 30 cycles of 94°C for 30 seconds, annealing for 30 seconds at 50°C, extension at 72°C for one and a half minutes, and a final extension at 72°C for seven minutes. PCRs were checked by running 5 μL of the reaction on a 1% agarose gel.

In most cases, a single band was obtained and purified using the Qiagen MinElute PCR Purification kit. In the event of multiple bands, the correct-sized fragment was excised from a 2% agarose gel over UV light and purified using QIAquick PCR purification spin columns. Forward and reverse strands were sequenced using sequencing services of Macrogen Inc., Korea (BigDyeTM terminator and ABI Sequencer 3730x, www.macrogen.com). Forward and reverse sequences were combined and checked for errors using ChromasPro Version 1.34 (Technelysium Pty Ltd). Final sequences were aligned in Clustal W using default parameters and adjusted by eye. Regions of ambiguous alignment were excluded and gaps were treated as missing.

2.4   *Phylogenetic analysis*

Following the principle of 'total evidence' (e.g., Prendini et al. 2003), the 16S, 18S, and 28S sequences were analyzed simultaneously. The combined sequences contained about 2.6 kilobases of nucleotide data. Maximum parsimony analyses (MP) were conducted in PAUP* 4.0b10 (Swofford 2002) (heuristic search, TBR, random addition sequence, 500 replicates). Initial analyses were conducted under equal character weights. Topological robustness was assessed using parsimony jackknifing (Farris et al. 1996). Jackknife frequencies were calculated in PAUP* using 1000 pseudoreplicates under a heuristic search with 30% character deletion.

Analyses using Bayesian inference (BI) were conducted in MrBayes Version 3.1.2 (Huelsenbeck & Ronquist 2001). Metropolis coupled Monte Carlo Markov Chains were run for 2,000,000 generations. Four differentially heated chains were run in each of two simultaneous runs. Topologies were sampled every 100 generations. Likelihood settings were determined during the run. Base frequencies were estimated, as were the rates of the six substitution types (nst = 6). A discrete gamma distribution was assumed for variation in the rate of substitution between nucleotide positions in the alignment, and the shape parameter of this distribution was estimated. After inspection of the likelihoods of the sampled trees, the first 50,000 generations were discarded as 'burn in.' All remaining topologies had likelihoods within 0.1% of the long-term asymptote in each run, suggesting that these were sampled after the Markov Chain's convergence to a stable posterior probability distribution. The standard deviation of split frequencies converged to a value of 0.004946. All trees remaining after discarding 'burn in' were used to calculate posterior probabilities using a majority rule consensus.

Table 2. Sequencing primers used.

Primer name	Sequence		Source
18S-F07	5' – CTG GTT GAT CCT GCC AG – 3'	18S PCR primer	Medlin et al. (1998)
18S-R1514	5' – TGA TCC TTY GCA GGT TCA C – 3'	18S PCR primer	Sogin (1990)
18S-R651	5' – CGA GGT CCT ATT CCA TTA TTC C – 3'	18S Sequencing primer	Newly designed herein
18S-F551	5' – GGT AAT TCG AGC TCC RRT AGC G – 3'	18S Sequencing primer	Newly designed herein
18S-F1053	5' – GAT TCT ATG GGT GGT GGT – 3'	18S Sequencing primer	Newly designed herein
28S-F216	5' – CTG AAT TTA AGC ATA TTA ATT AGK GSA GG – 3'	28S PCR & sequencing primer	Newly designed herein
28S-R443	5' – CCT CAC GGT ACT TGT TCG CTA TCG G – 3'	28S PCR & sequencing primer	Newly designed herein
LR-N-13398	5' – CGC CTG TTT AAC AAA AAC AT – 3'	16S forward PCR & sequencing primer	Morrison et al. (2002)
LR-J-12887	5' – CCG GTC TGA ACT CAG ATC ACG T – 3'	16S reverse PCR & sequencing primer	Morrison et al. (2002)

3   RESULTS

3.1   *Sequence data*

We collected 54 new sequences from 19 species (18 for 16S, 17 for 18S, and 19 for 28S) (GenBank accession numbers: EU821536, EU821532–821536, E821571–821581, EU831282–831286, EU834069). The aligned combined dataset contained 44 taxa and 2627, characters of which 795 are parsimony informative. The aligned 16S rRNA dataset contained 422 characters, of which 297 are variable (70%) and 216 are parsimony informative (51%). The aligned 18S rRNA dataset contained 1913 characters with 693 variable sites (36%), of which 450 are parsimony informative sites (24%). The aligned 28S rRNA dataset contained 292 characters, of which 170 are variable (58%) and 129 parsimony informative (44%). The 16S fragment is relatively AT rich compared to the other two fragments. Departures from base homogeneity, according to χ^2 tests of nucleotide composition for each gene fragment, were significant for 16S and insignificant for 18S and 28S (16S, df = 132, P = 0.55; 18S, df = 132, P = 1.00; 28S, df = 132, P = 1.00).

3.2   *Analyses: maximum parsimony and Bayesian inference*

MP analysis under equal weights retrieved a single, fully resolved topology of length (TL) 3836, consistency index (CI) 0.4726, retention index (RI) 0.6184 (Fig. 2). Hippoidea, containing *Emerita*, *Lepidopa*, and *Blepharipoda*, representing Hippidae, Lepidopidae, and Blepharipodidae, respectively, was monophyletic and sister to the remaining anomurans, corroborating Martin & Abele (1986), Pérez-Losada et al. (2002), Ahyong & O'Meally (2004), Porter et al. (2005), Macpherson et al. (2005), and Tsang et al. (2008). Galatheoidea and Paguroidea, however, are significantly polyphyletic. Three clades of paguroids, corresponding respectively to Diogenidae + Coenobitidae, Parapaguridae + *Trizocheles*, and Paguridae + *Pylocheles*, are widely dispersed. Notably, the two pylochelid terminals, *Pylocheles* and *Trizocheles*, are never in close proximity, instead being associated with Paguridae and Parapaguridae, respectively. *Lithodes* + *Oedignathus* (representing
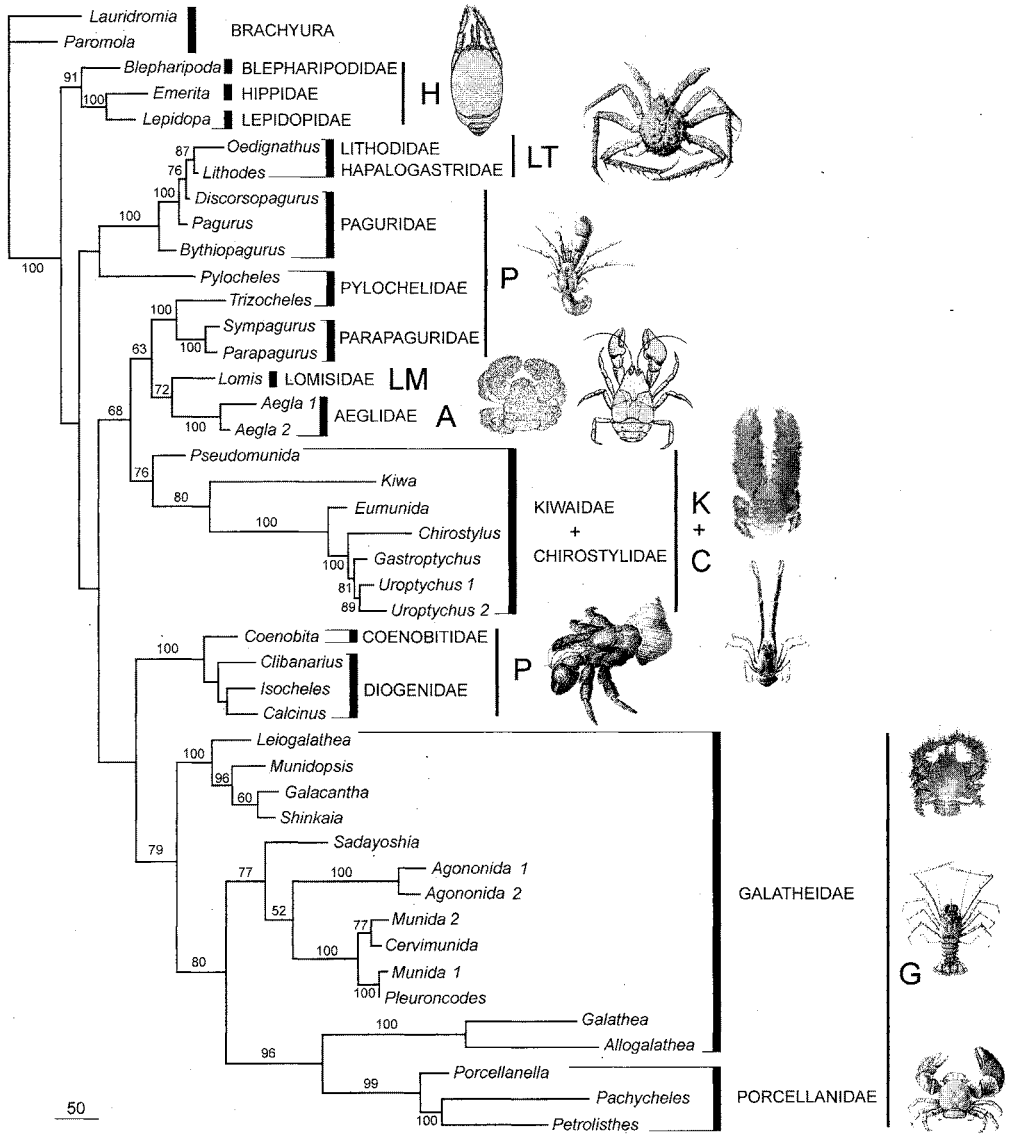
Figure 2. Phylogeny of Anomura. Single most parsimonious topology derived from MP analysis under equal weights (TL = 3836, CI = 0.4726, RI = 0.6184). Jackknife proportions indicated at nodes. Superfamilies as recognized by McLaughlin et al. (2007) abbreviated as follows: Aegloidea – A; Chirostylidae – C; Kiwaoidea – K; Galatheoidea – G; Hippoidea – H; Lithodoidea – LT; Lomisoidea – LM; Paguroidea – P.

Lithodidae + Hapalogastridae) is nested within Paguridae. The Paguridae + *Pylocheles* clade is sister to the major clade containing the remaining paguroids and galatheoids *sensu lato*. Aeglidae and Lomisidae are sister taxa, which together are sister to Parapaguridae + *Trizocheles*. The parapaguridaeglid-lomisid clade is sister to a monophyletic Chirostylidae (with the inclusion of *Kiwa*). Diogenidae is sister to Galatheidae + Porcellanidae. *Shinkaia* (representing Shinkaiinae), *Munidopsis* and *Galacantha* (representing Munidopsinae), and *Leiogalathea* (Galatheiinae) together form a clade that is sister to the remaining galatheids/porcellanids. Within this larger galatheid/porcellanid clade, Porcellanidae is deeply nested, rendering Galatheidae paraphyletic. Jackknife support for 'backbone' nodes was generally low, though clades corresponding to currently recognized families were usually strongly supported (Fig. 2).

Results of BI (Fig. 3) were compatible with, but 'basally' less resolved than, MP results. A hippoid clade, diogenid clade, galatheid + porcellanid clade, pagurid clade, and chirostylid-kiwaidparapagurid-lomisid-aeglid clade were all recovered with strong support (posterior probability 0.98 or higher). Notably, each of the paguroid clades was dispersed, as were the major galatheoid clades. As in MP results, the two pylochelid terminals were never associated and a monophyletic Porcellanidae nests within a paraphyletic Galatheidae. Under both MP and BI, the Galatheidae and Chirostylidae are not closely related to each other.

4 DISCUSSION

4.1 Polyphyly of Paguroidea and Galatheoidea

The most striking aspect of the present results is the radical polyphyly of Paguroidea and Galatheoidea. Despite ongoing controversy over internal interrelationships, general consensus has recognized three major clades corresponding to Hippoidea, Galatheoidea, and Paguroidea, irrespective of debate over the positions of one or other constituent groups (e.g., Lomisidae: McLaughlin 1983a; Aeglidae: Pérez-Losada et al. 2002, Ahyong & O'Meally 2004; and, more recently, Pylochelidae: Ahyong & O'Meally 2004). Present results retrieve well-supported clades of paguroids corresponding to Paguridae, Parapaguridae, and Diogenidae + Coenobitidae, respectively. Pylochelidae, however, represented by *Pylocheles* and *Trizocheles*, is not supported as monophyletic. Most significantly, a monophyletic Paguroidea is never recovered. MacDonald et al. (1957) questioned the monophyly of the paguroids based on larval characters, and Tudge (1997), using spermatozoal morphology, found Paguroidea not to be strictly monophyletic owing to incursion of galatheoids. Others, however, have cogently defended paguroid monophyly (McLaughlin 1983b; Richter & Scholtz 1994). Under BI, the positions of major clades of paguroids are either unresolved or dispersed to the proximity of the chirostylids-kiwaids-lomisids-aeglids. Under MP, however, topologies are fully resolved: one paguroid clade (Diogenidae) aligns with the galatheid + porcellanid clade; another (Parapaguridae + *Trizocheles*) forms a clade together with aeglids, lomisids, and chirostylids; and a third clade (Paguridae + Lithodidae + Hapalogastridae) is distant from both Galatheidae and Chirostylidae. Several of the nodes that are unresolved under BI are recovered by MP, but with low jackknife support. Exclusion of parapagurids + *Trizocheleles* from other paguroids is well supported, but the relationship among other paguroid clades is less clear. The pattern of paguroid polyphyly is thus difficult to interpret, though analyses are unequivocal in challenging a strictly monophyletic origin of the hermit crabs. That a monophyletic Pylochelidae is not recovered is perhaps not surprising — likely paraphyly has already been recognized (e.g., Richter & Scholtz 1994; McLaughlin et al. 2007). However, polyphyly of the asymmetrical hermit crabs is difficult to reconcile with somatic morphology. *A priori*, the suite of associated modifications required for gastropod shell habitation, present in all asymmetrical paguroids, is compelling evidence of monophyly. Significant convergence is implied if the hermit crabs are polyphyletic, with independent derivations of asymmetry in Paguridae, Coenobitidae + Diogenidae, and Parapaguridae. Such a scenario seems unlikely, though perhaps plausible, given the discovery that development of abdominal asymmetry
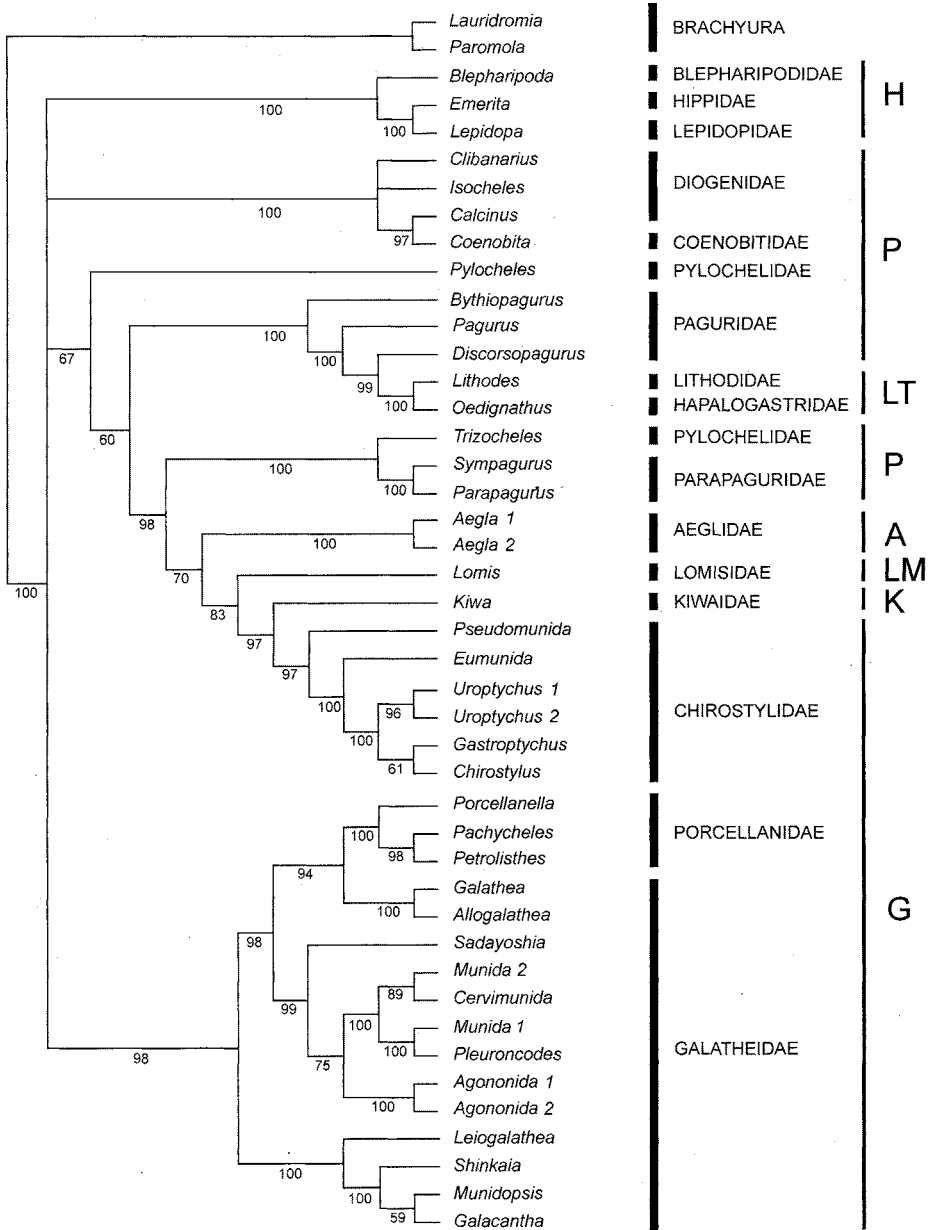
Figure 3. Phylogeny of Anomura. Bayesian topology; posterior probabilities indicated on branches as percentages. Superfamilies as recognized by McLaughlin et al. (2007) abbreviated as follows: Aegloidea – A; Kiwaoidea – K; Galatheoidea – G; Hippoidea – H; Lithodoidea – LT; Lomisoidea – LM; Paguroidea – P.

is mediated, at least in part, by environmental factors (Przibam 1907; Harvey 1998). It is also perhaps of more than passing interest that the asymmetrical hermit crab exemplars align basally with different paguroid clades, respectively (under MP: *Trizocheles* with Parapaguridae; *Pylocheles* with Paguridae). Our molecular data strongly corroborate monophyly of the three major paguroid clades (i.e., family level taxa), so the absence of molecular support for overall paguroid monophyly is significant. It should be noted, however, that important phylogenetic information could be contained in hypervariable regions that presently defy alignment and were excluded from the analysis. Also, taxon sampling within speciose families is limited, so a more extensive taxon set may influence topologies.

Galatheoidea, universally recognized to at least include the squat lobsters (Galatheidae and Chirostylidae) and porcelain crabs (Porcellanidae), is not supported as monophyletic. The chirostylids are well removed from the galatheids and porcellanids, being more closely related to an assemblage including aeglids, kiwaids, lomisids, and some hermit crabs. This wide phylogenetic separation, while unexpected, is not counterintuitive. As with Aeglidae, which was formerly assigned to Galatheoidea (e.g., Martin & Davis 2001), the remaining galatheoids have been thought related on the basis of overall habitus, having the generally elongated cephalothorax and 'long tail.' These features, however, are plesiomorphies, and little otherwise unites the galatheoid families. Indeed, McLaughlin et al. (2007) reported only a single unifying synapomorphy of Galatheoidea: the progressive development of the orbits. The orbital structure in galatheids, chirostylids, and porcellanids, though similar, appears to be linked to the well-developed rostrum, which is a plesiomorphy. Thus, given the absence of robust synapomorphies, the polyphyly of Galatheoidea is not surprising.

The 'hairy crab,' *Kiwa hirsuta* (Kiwaidae), was originally posited as sister to the Galatheidae + (Chirostylidae + Porcellanidae) clade with strongest morphological similarities to aeglids and chirostylids (Macpherson et al. 2005). These observations are consistent with present results in the close molecular relationship between chirostylids, aeglids, and kiwaids. Indeed, under MP, *Kiwa* is nested within Chirostylidae, albeit with moderate jackknife support, raising questions about the validity of Kiwaidae. *Kiwa* and chirostylids uniquely share the complete loss of the last thoracic sternite, which was initially regarded as a parallelism (Macpherson et al. 2005; McLaughlin et al. 2007) but is now more parsimoniously interpreted as a synapomorphy. That the chirostylids may be closer to non-galatheoids than galatheids or porcellanids is consistent with observations of other workers. Larval characters of *Chirostylus* are markedly dissimilar to larval *Galathea* (see Clark & Ng 2008), and chirostylid sperm morphology is more similar to that of hermit crabs than to other galatheoids (Tudge 1995, 1997).

Although aeglids are usually classified as galatheoids on the basis of general habitus, their affinities have been widely debated, notably with regards to paguroid affinities (Dana 1852; Martin & Abele 1988, 1986). Similarly, lomisoids have been variously treated as porcellanids, paguroids, or as independent (Pilgrim 1965; McLaughlin 1983a). The *Lomis* + *Aegla* clade recovered here under MP corroborates other recent studies based on mitochondrial gene rearrangements (Morrison et al. 2002), somatic morphology and molecular data (Ahyong & O'Meally 2004; Porter et el. 2005), and spermatozoal morphology (Tudge & Scheltinga 2002). Only very recently were aeglids formally removed to their own superfamily (McLaughlin et al. 2007).

Three subfamilies of Galatheidae are currently recognized (Baba & Williams 1998): Galatheinae, Munidopsinae, and Shinkaiinae. Representatives of the munidopsines (*Munidopsis* and *Galacantha*) and shinkaiines (*Shinkaia*) together with the galatheine, *Leiogalathea*, form a well-supported clade that is sister to the remaining galatheids/porcellanids. The position of *Leiogalathea* is unexpected, because it closely resembles other galatheines such as *Allogalathea* and *Galathea*. *Leiogalathea* thus warrants further scrutiny for morphological corroboration of molecular patterns. The close relationship between Galatheidae and Porcellanidae is widely recognized (e.g., McLaughlin et al. 2007), but the possibility that porcellanids are derived from within the galatheids is novel. The crab-like form of porcellanids, an example of carcinization within the Anomura, is derived.

However, the chief characters separating galatheids from porcellanids, namely the well-developed rostrum; deeper, more elongate cephalothorax; more muscular and more elongate abdomen; and anteriorly directed chelipeds, are plesiomorphic. Thus, derivation of Porcellanidae from within Galatheidae is morphologically plausible. Further studies with larger suites of both families are required to test the reciprocal monophyly implied by the current classification. *Munida* is not monophyletic under either BI or MP; the two exemplars are more closely related to *Cervimunida* or *Pleuroncodes*, respectively. With almost 250 known species of *Munida*, this result must be considered indicative only, though recent studies already suggest that *Munida* requires further division (e.g., Machordom & Macpherson 2004; Cabezas et al. 2008).

4.2 *Carcinization*

Borradaile (1916) first coined the term carcinization for evolution of the crab-like form, with the best known example being the derivation of king crabs (Lithodoidea: Lithodidae and Hapalogastridae) from within the asymmetrical hermit crabs. Derivation of the king crabs from within the paguroids has been widely supported by both molecular and morphological studies (e.g., Boas 1880; Bouvier 1894a–c, 1895 a, b; Cunningham et al. 1992; Richter & Scholtz 1994; McLaughlin et al. 1997; Morrison et al. 2002; Ahyong & O'Meally 2004; Tsang et al. 2008), though several recent studies dispute pagurid derivation of lithodids on the basis of apparently implausible transformation pathways (e.g., McLaughlin & Lemaitre 1997; McLaughlin et al. 2004, 2007). The 'hermit to king' hypothesis, however, is unequivocally corroborated here: Lithodidae + Hapalogastridae is nested within Paguridae. Independent carcinization events are also identified in the Porcellanidae and Lomisidae.

4.3 *Implications for anomuran classification*

The phylogenetic patterns recovered here are not compatible with recent anomuran classifications, either the four-superfamily system of Martin & Davis (2001) or the seven-superfamily system of McLaughlin et al. (2007). At the family level, few major problems are identified: polyphyly of Pylochelidae, paraphyly of Galatheidae with respect to Porcellanidae, and possible inclusion of Kiwaidae within Chirostylidae. The most significant and far-reaching challenges are in the likely polyphyly of the two largest superfamilies, Paguroidea and Galatheoidea. Of the superfamilies collectively recognized by Martin & Davis (2001) and McLaughlin et al. (2007), only Aegloidea, Hippoidea, and Lomisoidea remain uncontroversial from a nomenclatural perspective. Kiwaoidea and Lithodoidea are not compatible with present results. Lithodidae and Hapalogastridae are nested within Paguridae, rendering recognition of Lithodoidea problematical. *Kiwa* may be nested within Chirostylidae, which would preclude separate familial or superfamilial status for the former. Moreover, Chirostylidae itself is excluded from Galatheoidea and would warrant its own superfamily. Similarly, among the asymmetrical hermit crabs, Parapaguridae appears to be independent of the other major paguroid clades, also warranting superfamilial status. For the remaining major hermit crab clades, recognition of either one or two superfamilies is more ambiguous. The pagurid and diogenid + coenobitid clades are independent under MP, but nodal support for their separation is equivocal, so these potentially could constitute a monophylum. The current classification will require either abandonment of superfamilies or recognition of several more.

5 CONCLUSIONS

The internal phylogenetic relationships of the Anomura remain contentious, and consensus is still far off. The diversity of phylogenetic hypotheses proposed, even in the last two decades, highlights the complexity of the issue. The present analyses, based on the largest molecular dataset

for the Anomura analyzed to date, offer new perspectives on the issue. Results corroborate several previous studies in the basal position of Hippoidea (Martin & Abele 1986; Pérez-Losada et al. 2002; Ahyong & O'Meally 2004; Macpherson et al. 2005) but point to significant polyphyly in the two largest superfamilies, Galatheoidea and Paguroidea. Whereas previous cladistic analyses have identified anomalous positions for one or other galatheoid or paguroid taxa, all have recovered major clades that substantially correspond to Paguroidea, Galatheoidea, and Hippoidea (e.g., Martin & Abele 1986; Morrison et al. 2002; Ahyong & O'Meally 2004; McLaughlin et al. 2007). Thus, present results are a significant departure from predecessors in suggesting that the asymmetrical hermit crabs have a strongly polyphyletic origin. Similarly, the chirostylids are derived independently of galatheids/porcellanids. Although it would be premature to change the classification at this stage, the phylogenetic patterns recovered suggest significant changes will be required.

Some patterns recovered herein, while unexpected, are not counterintuitive — namely, polyphyly of Galatheoidea. Reconsideration of the unifying characters of Galatheoidea shows that the group lacks synapomorphies. To date, Galatheoidea has been recognized on the basis of plesiomorphies, so it is hardly surprising that it collapses under phylogenetic analysis. Likewise, at a lower taxonomic level, Galatheidae may be paraphyletic on the basis of an internally nested Porcellanidae, and a similar situation may be obtained for Chirostylidae with respect to Kiwaidae. The close relationship between aegloids, lomisoids, and parapagurids to chirostylids and kiwaids recovered here has precedence to various degrees in other studies and is an obvious focus of further research.

Other patterns recovered herein are both unexpected and counterintuitive — namely, polyphyly of the asymmetrical hermit crabs. Morphological synapomorphies unifying the Paguroidea are often complex and related to the almost universal habit of occupying gastropod shells. The apparent polyphyly of the paguroids suggests independent derivations of asymmetry in three separate clades: Paguridae, Coenobitidae + Diogenidae, and Parapaguridae. Such a result, however, should not be automatically dismissed. If carcinization can have multiple, independent origins (e.g., in Lithodoidea, Porcellanidae, Lomisoidea) (Morrison et al. 2002), then why not adaptation to dextral shell habitation? Much of the recent debate in anomuran phylogenetics is over the reality of carcinization and revolves around the position of lithodids with respect to the hermit crabs. However, present results pose even more fundamental questions about whether the Paguroidea is even a natural group.

Clearly, further research is required using more taxa and more data; available data sources, both morphological and molecular, are certainly far from exhausted. To this end, further investigations are currently underway, combined with morphological data and an expanded taxon set focused on the galatheoids. Nevertheless, the phylogenetic patterns suggested here ought to stimulate closer scrutiny of morphology, especially for unrecognized synapomorphies that could corroborate (or further challenge) unexpected molecular results. Ultimately, morphological plausibility is the criterion by which molecular phylogenetic hypotheses are evaluated, though that is not to say that morphology is yet fully understood.

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