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Hermit to King, or Hermit to All: Multiple Transitions to Crab-like Forms from Hermit Crab Ancestors

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Abstract.—The Anomura presents the greatest degree of morphological disparity in the decapod Crustacea, with body forms ranging from the symmetrical and asymmetrical hermit crabs to squat lobsters and king crabs. The phylogeny of the anomurans has been fraught with controversy. Recent debate has focused primarily on the phenomenon of carcinization, the evolution of crab-like form from a non–crab-like ancestor, focused chiefly on derivation of king crabs from asymmetrical hermit crabs—the "hermit to king" hypothesis. We show by phylogenetic analysis of five nuclear protein-coding gene sequences that hermit crabs have a single origin, but surprisingly, that almost all other major clades and body forms within the Anomura, are derived from within the hermit crabs. The crab-like form and squat lobster form have each evolved at least twice from separate symmetrical hermit crab ancestors. In each case, a carcinization trend can be posited via a transition series from the initial symmetrical long-tailed hermit crab form, through the intermediate squat lobster or asymmetrical hermit crab form, to the final crab-like form. Adaptation to dextral shell habitation evolved at least twice, once in an exclusively deep-water clade and once in the common ancestor of all other asymmetrical hermit crabs (from which king crabs are derived). These remarkable cases of parallelism suggest considerable phenotypic flexibility within the hermit crab ground plan, with a general tendency toward carcinization. Rather than having a separate origin from other major clades, hermit crabs have given rise to most other major anomuran body types. [Anomura; Decapoda; Paguroidea; carcinization; data partition; phylogeny; parallel evolution.]

The 17,600+ species of decapod crustaceans are presently distributed in 10 infraordinal clades (De Grave et al. 2009). These clades include such familiar crustaceans as the shrimps and prawns (Caridea and Dendrobranchiata), lobsters and freshwater crayfish (Astacidea), the true crabs (Brachyura), and the hermit and king crabs (Anomura). Previous phylogenetic analyses indicate that the anomurans are the sister group of the true crabs, collectively termed, Meiura (Scholtz and Richter 1995). Of all decapod clades, the Anomura presents the greatest degree of morphological disparity. Anomura includes not only the hermit and king crabs, but also marine and freshwater squat lobsters, porcelain crabs, and fossorial mole crabs (McLaughlin et al. 2007; Ahyong et al. 2009; McLaughlin, Boyko, et al. 2010; Fig. 1). The most familiar anomurans are probably the hermit crabs, Paguroidea, with more than 1000 species (De Grave et al. 2009; McLaughlin, Komai, et al. 2010). They occur in shallow waters, the deep sea, and even on land. Hermit crabs are so named because they usually use a gastropod shell or other hollow object to protect their hind body (pleon), which in most species is soft and asymmetrically coiled to fit coiled gastropod shells (Fig. 1a–e). Conversely, some putatively more "primitive" hermit crabs (family Pylochelidae) have a more highly calcified and symmetrical pleon (Fig. 1j-l). They usually live in pieces of hollow wood or straight worm tubes instead of coiled gastropod shells. The squat lobsters (Fig. 1n–q) are less familiar than hermit crabs but are almost as diverse, with about 900 known species (Baba et al. 2008). These include the freshwater squat lobsters (Aeglidae) (Fig. 1q), yeti crab (Kiwaidae) (Fig. 1n) from hydrothermal vents, coral associated Chirostylidae (Fig. 1o), and generally free-living Galatheidae (Fig. 1p) (Baba et al. 2008). Squat lobsters share an elongated pleon that is held partially folded under the body—they have a somewhat lobster-like body form, hence their common name. Some anomurans are distinctly crab-like; they have a broadened carapace and sternum, and a reduced pleon that is fully folded beneath the body as in true crabs (Brachyura): these are the king crabs (Lithodidae) (Fig. 1r), porcelain crabs (Porcellanidae) (Fig. 1s), and hairy stone crabs (Lomisidae) (Fig. 1t). The fossorial mole crabs (Hippoidea) (Fig. 1m) have the pleon partially folded under the body with a general appearance very similar to some primitive brachyuran crabs (e.g., the frog crabs, Raninoida).

Not surprisingly, the evolution and phylogeny of the anomurans have been surrounded by controversy (Ahyong and O'Meally 2004; McLaughlin et al. 2004; McLaughlin et al. 2007; McLaughlin, Boyko, et al. 2010; McLaughlin, Komai, et al. 2010; Ahyong et al. 2009). The hermit crabs are unusual in using portable, hollow domiciles to protect the pleon (unique in Decapoda but not unique in Crustacea), and in the presence of pleonal rather than cephalothoracic midgut caeca (unique in Anomura but not unique in Decapoda). Recent debate over anomuran phylogeny has focused primarily on the phenomenon of carcinization. "Carcinization" was

first coined by Borradaile (1916) in reference to aspects of morphology of the hermit crab *Porcellanopagurus*, but it is now widely understood to denote derivation of a crab-like body form from a non-crab ancestor in clades outside of the Brachyura (the true crabs) (Wolff 1961; Guinot 1979; Cunningham et al. 1992; Morrison et al. 2002; Ahyong et al. 2009). Essentially, carcinization is achieved through widening of the carapace and thoracic sternum, and shortening and reduction of the pleon, which is held fully folded flat under the body. Also, the chelipeds, which are plesiomorphically directed forward, can be folded transversely across the anterior of the cephalothorax. The focus of most carcinization debates has been on whether or not the king crabs were derived from within the asymmetrical hermit crabs, the "hermit to king" hypothesis (Cunningham et al. 1992). Recent molecular phylogenetic studies, however, not only support the hermit to king hypothesis, but also suggest that asymmetry in hermit crabs may have multiple origins and that convergence of body form may be significantly more prevalent than previously recognized (Ahyong et al. 2009; Bracken et al. 2009; Chu et al. 2009). Accordingly, evaluation of the origins and pathways of carcinization could provide important insights into the evolution and adaptation in this morphologically and ecologically diverse group of animals. Evaluation of carcinization hypotheses naturally requires robust knowledge of phylogeny.

Numerous morphological and molecular studies (e.g., Cunningham et al. 1992; Morrison et al. 2002; Tsang et al. 2008; Ahyong et al. 2009; Chu et al. 2009) support the hermit to king hypothesis and in some cases suggest hermit crab polyphyly. These hypotheses, however, are strongly opposed by some larval and adult morphological studies that reject asymmetrical hermit crab ancestry of king crabs (McLaughlin and Lemaitre 1997; McLaughlin et al. 2004, 2007). Previous efforts to elucidate the anomuran phylogeny, based exclusively on morphology, or mitochondrial DNA (mtDNA) and rDNA sequence data have suffered from insufficient topological robustness or taxon sampling to draw strong conclusions. To evaluate the evolution of Anomura, we generated a molecular data set with >2600 bp of DNA sequence from five nuclear protein-coding gene regions across 14 of 17 recognized families. The phylogenetic relationships are well resolved at most nodes. We further mapped different morphological forms of anomurans onto the inferred phylogeny in order to reconstruct the history of body form transitions.

Materials and Methods

Taxon Sampling and Sequencing

A total of 46 species spanning 14 of the 17 anomuran families and five of the six hermit crab families (with the exception of monotypic Pylojacquesidae) were included in our study (Table 1). We followed the most recent classification scheme of De Grave et al. (2009). Total genomic DNA was extracted from the pleopods or pereiopods of the anomuran species and the four outgroup taxa (Table 1) using the commercial QIAamp Tissue Kit (Qiagen). Primers for amplifying the five genes, *arginine kinase* (*AK*), *enolase*, *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*), *sodium potassium ATPase* α-*subunit* (*NaK*), and *phosphoenolpyruvate carboxykinase* (*PEPCK*), are listed in Table 2. The amplifications were conducted in a reaction mix containing 1–5 μL of template DNA, 1× polymerase chain reaction (PCR) reaction buffer, 3 mM $MgCl_2$, 200 nM of each primer, 200 μM dNTPs, 1.5 units of *Taq* polymerase (Qiagen) and ddH_2O to a total volume of 25 μL. The PCR profiles were as follows: 3 min at 94°C for initial denaturation, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50–60°C (depending on the primers and taxa) for 1 min, elongation at 72°C for 1.5 min, and a final extension at 72°C for 10 min. The PCR products were then purified using the QIAquick gel purification kit (Qiagen) according to manufacturer's instructions. Sequencing reactions were carried out using the same sets of primers and the ABI Big-dye Ready-Reaction mix kit, following the standard cycle sequencing protocol. The products were analyzed using an Applied Biosystems (ABI) 3100 automated sequencer.

Phylogenetic Analyses

Sequences were aligned using CLUSTAL W (Thompson et al. 1994) with default parameters and confirmed by translating into amino acid sequences. The total data set was analyzed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses. MP analysis was performed using heuristic search and tree-bisection-reconnection with 1,000 random addition sequence replicates on PAUP*4.0b10 (Swofford 2002). Character states were unordered and equally weighted. Gaps were treated as missing data. Bootstrap (BP) support for the most parsimonious tree was evaluated using 1000 replicates with 100 random sequence addition replicates. We used Modeltest 3.7 (Posada and Crandall 1998) to select the best-fit models of nucleotide substitution for each data set based on the Akaike information criterion (Akaike 1974). Partitioning of sequence data from multiple genes under a mixed model can offer better estimation of phylogenetic relationships (Castoe et al. 2004; Brandley et al. 2005; Brown and Lemmon 2007). Therefore, we attempted to test and employ various partition schemes for the ML and BI analyses to determine the best fitting partition scheme and phylogenetic inference. We divided the data set into different partitions according to their biological function (i.e., gene and/or codon position). As the first and second codon positions are expected to exhibit more similar substitution properties compared with the third codon position (Kimura 1980; Nei 1987), we also tried to combine the two into one partition

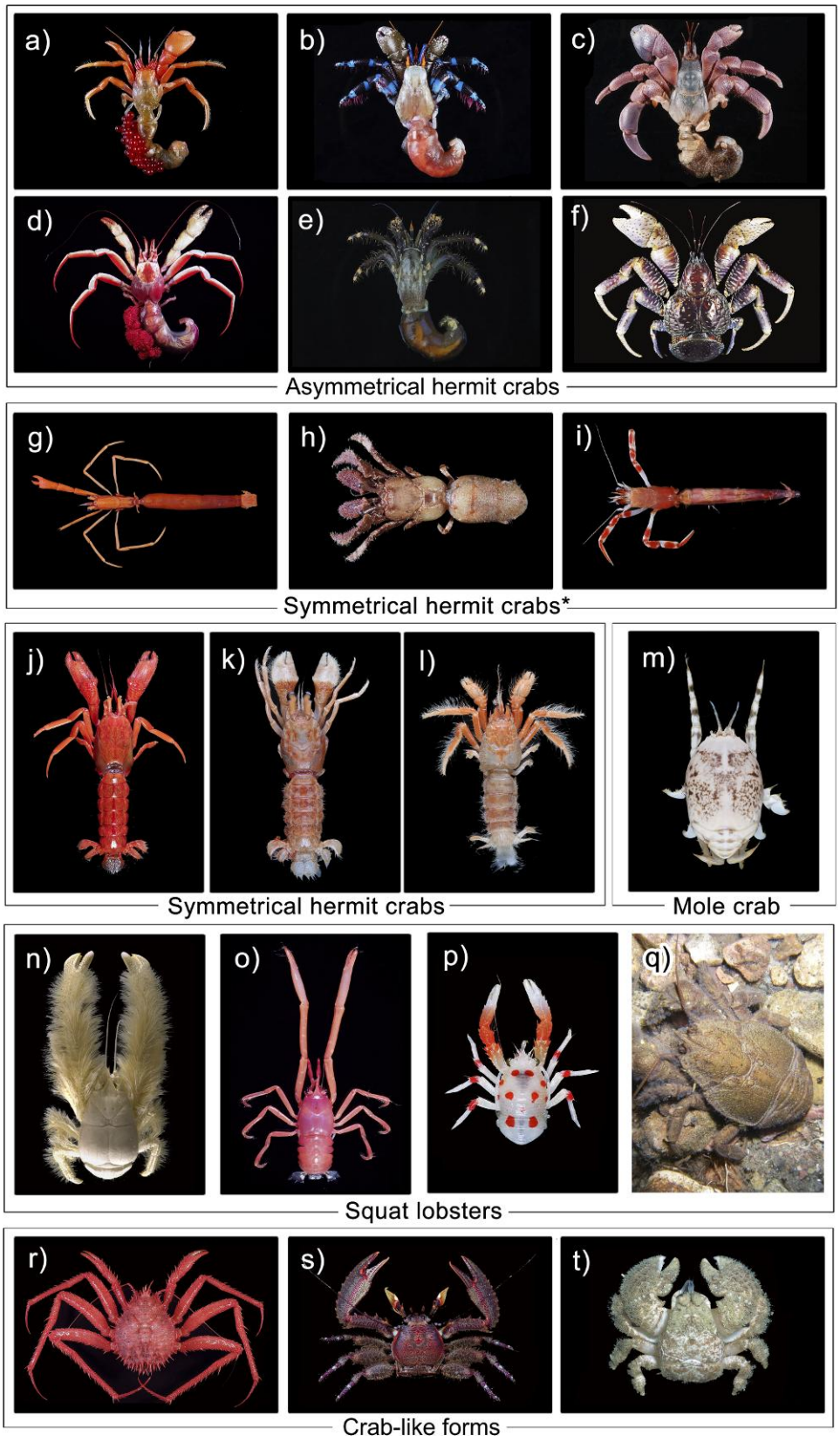

FIGURE 1.

while the third codon position is separated into another partition. We employed a total of five different analytical schemes: (i) all data combined without partitions (partition scheme P1); (ii) partitioned by three different codon positions (P3); (iii) partitioned by gene (P5); (iv) partitioned by gene and the first + second and the third codon positions (P10); and (v) partitioned by gene and three codon positions (P15) (Table 3). ML analysis was implemented with RAxML 7.0.3 (Stamatakis 2006). The model GTRGAMMAI was used for different partitions, with individual α-shape parameters, GTR rates and base frequencies estimated and optimized for each partition. We conducted 1000 BP runs and searched for the best scoring ML tree. Bayesian analysis was performed using MrBayes v.3.12 (Ronquist and Huelsenbeck 2003). In some circumstances where the model determined by ModelTest for some partitions was not available in MrBayes, we used the most similar model available in MrBayes (Table 4). Two independent runs were carried out with four differentially heated Metropolis coupled Monte Carlo Markov Chains for 10,000,000 generations started from a random tree. Model parameters were estimated during the analysis. Chains were sampled every 1000 generations. Convergence of the analyses was validated by the standard deviation of split frequencies and monitoring of the likelihood values over time graphically using Tracer v1.4 (Rambaut and Drummond 2007). The trees generated prior to the achievement of stationarity of the log-likelihood values (5000 trees) were discarded as burn-in. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PP). The harmonic mean of the negative log likelihood was computed for each BI analysis by MrBayes. The values were used to calculate the Bayes factor (BF), which is twice the difference in the harmonic mean −lnL scores (Nylander et al. 2004), to compare the performance of different partition schemes as suggested by some studies (Brandley et al. 2005; Brown and Lemmon 2007). We evaluated alternative hypotheses according to the framework provided by Kass and Raftery (1995).

Alternative a priori phylogenetic hypotheses from previous morphological and molecular studies were tested using the likelihood-based Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) implemented in PAUP*, and by comparing the BF between the unconstrained topology obtained from the Bayesian analysis with those under the constraint of a priori phylogenetic hypotheses (Nylander et al. 2004; Brandley et al. 2005). Alternative tree topologies were constructed using MacClade 3.0 (Maddison W.P. and Maddison D.R. 1992) by rearranging the branches showing conflicting relationships between the inferred topology and the a priori hypotheses. The SH test was carried out with RELL optimization and 1000 BP pseudoreplicates. Specifically, we tested the hypothesis of reciprocal monophyly of the two superfamilies, Galatheoidea and Paguroidea, which have been long and widely accepted (e.g., Martin and Davis 2001; McLaughlin et al. 2007; De Grave et al. 2009), but which was rejected by our molecular phylogeny. We also tested for a derivation of the king crab from a hermit crab ancestor—the hermit to king hypothesis (Cunningham et al. 1992; Morrison et al. 2002).

We reconstructed the pattern of body form evolution of anomurans by mapping the four different body forms: symmetrical hermit crab, asymmetrical hermit crab, squat lobster and crab-like onto the inferred phylogeny using ML approaches described by Pagel (1999) implemented in BayesTraits v1.0 (available at www.evolution.reading.ac.uk). This approach is often preferred over parsimony-based methods, which do not consider branch lengths and models of nucleotide evolution (Cunningham et al. 1998). The likelihood of different possible ancestral states of the nodes was also estimated.

Results

The combined data set consisted of 2664 bp from five gene fragments (Table 4). A 3-bp insertion was observed in the *GADPH* gene of *Kiwa hirsuta* and *Sympagurus burkenroadi*, and a 3-bp deletion was found in the *NaK* gene of *Hippa adactyla* and *Icelopagurus crosnieri*. All these deletions/insertions did not represent frameshift mutations. The empirical base frequencies, model selected/employed for each partition are shown in Table 4. (TreeBASE study accession no. S11410, matrix accession no. M3827.)

The phylogenetic analyses based on the five partition schemes using ML and Bayesian methods resulted in three different tree topologies (Fig. 2 for Topology A, and Topologies A–C of Fig. 3). In general, the topology generated from ML and BI analyses are highly congruent for the same partitioning scheme, except when the data set is split into 10 partitions (P10). The unpartitioned data set (partition scheme P1) and partition by gene (partition scheme P5) support the same topology, which is also consistent with the topology from MP analysis (Fig. 2 and Topology A of Fig. 3). The BI analysis of partition scheme P10 also converges on this topology,

←
FIGURE 1. Body forms and morphological diversity of Anomura. a) *Bathypaguropsis kuroshioensis* (Paguridae). b) *Calcinus elegans* (Diogenidae). c) *Coenobita rugosus* (Coenobitidae). d) *Sympagurus burkenroadi* (Parapaguridae). e) *Clibanarius virescens* (Diogenidae). f) *Birgus latro* (Coenobitidae). g) *Xylopagurus philippinensis* (Paguridae). h) *Cancellis panglaoensis* (Diogenidae). i) *Tsunogaipagurus chuni* (Parapaguridae). j) *Xylocheles macrops* (Pylochelidae: Pylochelinae). k) *Pylocheles mortensenii* (Pylochelidae). l) *Trizocheles sakaii* (Pylochelidae). m) *Hippa marmorata* (Hippidae). n) *Kiwa hirsuta* (Kiwaidae). o) *Uroptychus orientalis* (Chirostylidae). p) *Galathea rubromaculata* (Galatheidae). q) *Aegla neuquensis* (Aeglidae). r) *Neolithodes nipponensis* (Lithodidae). s) *Petrolisthes coccineus* (Porcellanidae). t) *Lomis hirta* (Lomisidae). * These hermit crabs belong to the families predominately having asymmetrical abdomen and possess asymmetrical pleopods and uropods, in spite of their symmetrical abdomen.

TABLE 1. Classification, sampling locations and voucher ID of the species and GenBank accession number of the gene sequences of the present study

Superfamily	Family	Species	Sampling location	Voucher ID	Gene				
					AK	*enolase*	*GAPDH*	*NaK*	*PEPCK*
Aegloidea	Aeglidae	*Aegla alacalufi*	Argentina	Unvouchered	**GU382856**	**GU382903**	**GU382953**	**GU383035**	**GU383002**
	Aeglidae	*A. neuquensis*	Argentina	Unvouchered	**GU382857**	**GU382904**	**GU382954**	**GU383036**	**GU383003**
Galatheoidea	Chirostylidae	*Uroptychodes grandirostris*	Taiwan	NTOU A01103	**GU382898**	**GU382948**	**GU382997**	**GU383067**	**GU383034**
	Chirostylidae	*Eumunida funambulus*	Taiwan	NTOU A01104	**GU382866**	**GU382913**	**GU382963**	**GU383041**	**GU383008**
	Galatheidae	*Cervimunida princeps*	Taiwan	MSLKHC-Crpri	**GU382859**	**GU382906**	**GU382956**	**GU383037**	**GU383004**
	Galatheidae	*Munida albiapicula*	Taiwan	NTOU A00837	**GU382874**	**GU382921**	**GU382971**	EU427119	EU427188
	Galatheidae	*Munidopsis formosa*	Taiwan	NTOU A01105	**GU382872**	**GU382919**	**GU382969**	**GU383046**	**GU383013**
	Galatheidae	*Paramunida tricarinata*	Taiwan	NTOU A00838	**GU382886**	**GU382935**	**GU382984**	EU427122	EU427191
	Galatheidae	*Shinkaia crosnieri*	Okinawa	NTOU A01106	**GU382892**	**GU382942**	**GU382991**	**GU383061**	**GU383028**
	Porcellanidae	*Neopetrolisthes maculatus*	Aquarium shop, Hong Kong	NTOU A00928	**GU382876**	**GU382923**	**GU382973**	**GU383048**	**GU383015**
	Porcellanidae	*Petrolisthes japonicus*	Hainan, China	MSLKHC-Ptjap	**GU382889**	**GU382938**	**GU382987**	EU427123	EU427192
	Porcellanidae	*Novorostrum indicum*	Taiwan	NTOU A01006	**GU382877**	**GU382924**	**GU382974**	**GU383049**	**GU383016**
Hippoidea	Albuneidae	*Albunea occulta*	Taiwan	NTOU A00839	**GU382858**	**GU382905**	**GU382955**	EU427113	EU427182
	Hippidae	*Hippa adactyla*	Taiwan	NMNS 4368-027	**GU382867**	**GU382914**	**GU382964**	**GU383042**	**GU383009**
Kiwaoidea	Kiwaidae	*Kiwa hirsuta*	South East Pacific Rise	MNHN-Ga5310	**GU382869**	**GU382916**	**GU382966**	**GU383044**	**GU383011**
Lithodoidea	Lithodidae	*Lithodes turritus*	Taiwan	NTOU A01107	**GU382871**	**GU382918**	**GU382968**	**GU383045**	**GU383012**
	Lithodidae	*Neolithodes nipponensis*	Taiwan	NTOU A00845	**GU382875**	**GU382922**	**GU382972**	EU427120	EU427189
	Lithodidae	*Paralomis arae*	Taiwan	NTOU A00849	**GU382884**	**GU382933**	**GU382982**	n.a.	**GU383023**
	Lithodidae	*P. dofleini*	Taiwan	NTOU A01096	**GU382888**	**GU382937**	**GU382986**	**GU383059**	**GU383026**
Lomisoidea	Lomisidae	*Lomis hirta*	South Australia	NTOU A00840	**GU382870**	**GU382917**	**GU382967**	EU427118	EU427187
Paguroidea	Coenobitidae	*Coenobita rugosus*	Taiwan	NTOU A00842	**GU382863**	**GU382910**	**GU382960**	EU427116	EU427185
	Coenobitidae	*C. violascens*	Taiwan	NTOU A00841	**GU382862**	**GU382909**	**GU382959**	EU427115	EU427184
	Diogenidae	*Calcinus laevimanus*	Taiwan	NTOU A01100	**GU382860**	**GU382907**	**GU382957**	**GU383038**	**GU383005**
	Diogenidae	*Clibanarius virescens*	Hong Kong	NTOU A01092	**GU382861**	**GU382908**	**GU382958**	**GU383039**	**GU383006**
	Diogenidae	*Cl. humilis*	Philippines	NTOU A01094	n.a.	**GU382929**	**GU382978**	**GU383054**	**GU383021**
	Diogenidae	*Cl. englaucus*	Philippines	NTOU A01095	**GU382881**	**GU382930**	**GU382979**	**GU383055**	n.a.
	Diogenidae	*Dardanus impressus*	Taiwan	NTOU A00843	**GU382864**	**GU382911**	**GU382961**	EU427117	EU427186
	Diogenidae	*D. setifer*	Hong Kong	NTOU A01108	**GU382865**	**GU382912**	**GU382962**	**GU383040**	**GU383007**
	Paguridae	*Icelopagurus crosnieri*	Taiwan	NTOU A01099	**GU382868**	**GU382915**	**GU382965**	**GU383043**	**GU383010**
	Paguridae	*Michelopagurus limatulus*	Taiwan	NTOU A01098	**GU382873**	**GU382920**	**GU382970**	**GU383047**	**GU383014**
	Paguridae	*Pagurodofleinia doederleini*	Taiwan	NTOU A00846	**GU382883**	**GU382932**	**GU382981**	EU427114	EU427183
	Paguridae	*Pagurus angustus*	Taiwan	NTOU A01097	**GU382880**	**GU382927**	n.a.	**GU383052**	**GU383019**
	Paguridae	*Pagurus bernhardus*	Scotland	NTOU A01091	**GU382882**	**GU382931**	**GU382980**	**GU383056**	**GU383022**
	Paguridae	*Pagurus* sp.	Hong Kong	NTOU A01093	n.a.	**GU382928**	**GU382977**	**GU383053**	**GU383020**
	Paguridae	*Propagurus obtusifrons*	Taiwan	NTOU A00847	**GU382890**	**GU382940**	**GU382989**	EU427124	EU427193
	Paguridae	*Spiropagurus profundorum*	Taiwan	NTOU A00719	**GU382893**	**GU382943**	**GU382992**	**GU383062**	**GU383029**
	Parapaguridae	*Oncopagurus orientalis*	Taiwan	NTOU A00371	**GU382878**	**GU382925**	**GU382975**	**GU383050**	**GU383017**
	Parapaguridae	*Paragiopagurus acutus*	Philippines	NTOU A01110	**GU382879**	**GU382926**	**GU382976**	**GU383051**	**GU383018**
	Parapaguridae	*Paragiopagurus boletifer*	Taiwan	NTOU A00299	**GU382887**	**GU382936**	**GU382985**	**GU383058**	**GU383025**
	Parapaguridae	*Paragiopagurus ventilatus*	Taiwan	NTOU A01111	**GU382885**	**GU382934**	**GU382983**	**GU383057**	**GU383024**
	Parapaguridae	*Strobopagurus gracilipes*	Taiwan	NTOU A00009	**GU382894**	**GU382944**	**GU382993**	**GU383063**	**GU383030**
	Parapaguridae	*Sympagurus burkenroadi*	Taiwan	NTOU A00221	**GU382895**	**GU382945**	**GU382994**	**GU383064**	**GU383031**
	Pylochelidae	*Xylocheles macrops*	Taiwan	NTOU A00848	**GU382891**	**GU382941**	**GU382990**	EU427125	EU427194
	Pylochelidae	*Pylocheles mortensenii*	Taiwan	NTOU A01101	n.a.	**GU382939**	**GU382988**	**GU383060**	**GU383027**
	Pylochelidae	*Trizocheles brachyops*	New Zealand	NTOU A01109 (exNIWA)	**GU382896**	**GU382946**	**GU382995**	**GU383065**	**GU383032**
	Pylochelidae	*Trizocheles sakaii*	Taiwan	NTOU A01102	**GU382897**	**GU382947**	**GU382996**	**GU383066**	**GU383033**
Cancroidea	Cancridae	*Cancer japonicus*	Taiwan	NTOU B00003	**GU382899**	**GU382949**	**GU382998**	EU427127	EU427196
Dromioidea	Dromiidae	*Conchoecetes artificiosus*	Taiwan	NTOU B00005	**GU382900**	**GU382950**	**GU382999**	EU427129	EU427198
Grapsoidea	Varunidae	*Eriocheir japonica*	Hong Kong	MSLKHC-EjapHK	**GU382901**	**GU382951**	**GU383000**	EU427132	EU427201
	Upogebiidae	*Austinogebia edulis*	Hong Kong	MSLKHC-AeduHK	**GU382902**	**GU382952**	**GU383001**	EU427167	EU427236

New sequences are indicated in bold and "n.a." denotes missing sequence data.

TABLE 2. Primer sequences used for PCR amplification

Gene/primer	Sequence (5′ to 3′)	Source
Arginine kinase		
AK for a-1	CTC CCC TST TTG AYC CCA TCA T	This study
AK for a-2	ACC CCA TCA TTG AGG AYT AYC A	This study
AK for b	ATA GAC GAC CAC TTC CTS TTC AA	This study
AK rev 1	TGG AAC TCA GTC AGA CCC ATR CG	This study
AK rev 2	CCG CCC TCA GCC TCR GTG TGY TC	This study
Enolase		
Enol EA1	CAG CAA TCA ATG TCA TCA AYG GWG G	This study
Enol EA2	AGT TGG CTA TGC AGG ART TYA TGA T	This study
Enol ES1	ACT TGG TCA AAT GGR TCY TCA AT	This study
Enol ES2	ACC TGG TCG AAT GGR TCY TC	This study
Glyceraldehyde 3-phosphate dehydrogenase		
GAPDH F2	ATG AAG CCA GAA AAC ATT CCA TGG	This study
GAPDH GA	ATG GTG TAT ATG TTC AAG TAY GAY TC	This study
GAPDH R	GAA TAG CCT AAC TCG TTG TCR TAC CA	This study
GAPDH GR	TCG CTA GAT ACA ACA TCA TCY TCR GT	This study
Phosphoenolpyruvate carboxykinase		
PEPCK for	GTA GGT GAC GAC ATT GCY TGG ATG AA	Tsang et al. (2008)
PEPCK for2	GCA AGA CCA ACC TGG CCA TGA TGA C	Tsang et al. (2008)
PEPCK rev	GAA CCA GTT GAC GTG GAA GAT C	Tsang et al. (2008)
PEPCK rev3	CGG GYC TCC ATG CTS AGC CAR TG	Tsang et al. (2008)
Sodium potassium ATPase α-subunit		
NaK for-a	GTG TTC CTC ATT GGT ATC ATT GT	Tsang et al. (2008)
NaK for-b	ATG ACA GTT GCT CAT ATG TGG TT	Tsang et al. (2008)
NaK rev	ACC TTG ATA CCA GCA GAT CGG CAC TTG GC	Tsang et al. (2008)
NaK rev2	ATA GGG TGA TCT CCA GTR ACC AT	Tsang et al. (2008)

whereas the ML tree is slightly different in the arrangement of the position of Diogenidae and several internal nodes (Topology B in Fig. 3; see Supplementary Materials available at http://www.sysbio.oxfordjournals.org/). However, when we increased the number of partitions, the topology further changed. Partition scheme P3 (by codon position) and P15 (by both gene and codon position) resulted in another topology (Topology C). In sum, the three topologies differ chiefly in the position of the family Diogenidae and some internal nodes within Paguridae, whereas the relationships among other lineages remain stable. The likelihood score of the analysis that was partitioned by codon position was significantly better than analyses that were unpartitioned or partitioned only by gene (Table 3); the difference in likelihood scores between the latter two was relatively minor. BF comparisons favor the use of the full partitioning scheme (partition scheme P15), which also exhibits the highest likelihood score (Table 3).

The common method for calculation of BF using harmonic mean often overestimate the marginal likelihood and as a result, favors the choice of parameter rich partition scheme (Lartillot and Philippe 2006; Xie et al. 2011). Yet, it is widely acknowledged that overpartitioning would lead to error in phylogenetic inference (Sullivan and Joyce 2005; McGuire et al. 2007; Li et al. 2008). This may be a particular problem in the present study because many of the P15 partitions are small. Consequently, the number of observed transitions of some types may be so low that it becomes difficult to infer some entries in the rate matrix. Therefore, we intended to use the partition scheme that could optimize topology and PP/BP values, instead of justifying the suitability of the partition scheme based only on likelihood. The partition scheme P5 (partitioning by gene) performed the best in this regard, and most of the interfamilial relationships were highly resolved in both ML and BI analyses. Therefore, we adopted the partition scheme P5 in subsequent analyses and present the results from ML, BI, and

TABLE 3. Comparison of harmonic mean of -log likelihood and average standard deviation of split frequencies of two independent runs of BI analysis

Partition scheme	Number of partitions	Harmonic mean of log likelihood	Split deviation
P1: no partition	1	−30,512.10	0.003227
P3: by codon	3	−3,029,330.34	0.001784
P5: by gene	5	−3,030,470.70	0.002543
P10: Individual gene and 1st + 2nd and 3rd codon positions	10	−3,029,388.60	0.023804
P15: Individual gene and 1st, 2nd and 3rd codon positions	15	−3,029,232.27	0.010087

TABLE 4. Characteristics of the five molecular markers. The empirical base frequencies, substitution model selected by ModelTest and employed in Bayesian analyses of all partitions are listed

	No. of sites	No. of variable sites	No. of parsimony informative sites	A	C	G	T	Model chosen by ModelTest	Model implemented in MrBayes
AK 1st codon	210	42	29	0.25	0.25	0.32	0.18	TVM+I+G	GTR+I+G
AK 2nd codon	210	27	21	0.32	0.19	0.20	0.29	GTR+I+G	GTR+I+G
AK 3rd codon	210	179	162	0.04	0.49	0.28	0.19	GTR+I+G	GTR+I+G
AK 1st +2nd codons	420	69	50	0.29	0.22	0.26	0.23	GTR+I+G	GTR+I+G
AK	630	248	212	0.20	0.31	0.27	0.22	GTR+I+G	GTR+I+G
Enolase 1st codon	113	24	16	0.32	0.14	0.38	0.15	TVM+I+G	GTR+I+G
Enolase 2nd codon	113	16	8	0.38	0.19	0.15	0.28	F81+I	F81+I
Enolase 3rd codon	113	108	104	0.09	0.37	0.23	0.31	GTR+I+G	GTR+I+G
Enolase 1st +2nd codons	226	40	24	0.36	0.17	0.27	0.20	TVM+I+G	GTR+I+G
Enolase	339	148	128	0.26	0.23	0.25	0.25	GTR+I+G	GTR+I+G
GAPDH 1st codon	178	33	26	0.29	0.14	0.41	0.16	GTR+I+G	GTR+I+G
GAPDH 2nd codon	178	18	13	0.26	0.32	0.15	0.27	GTR+I	GTR+I
GAPDH 3rd codon	178	166	158	0.11	0.31	0.23	0.35	GTR+I+G	GTR+I+G
GAPDH 1st +2nd codons	356	51	39	0.27	0.24	0.28	0.21	TVMef+I+G	SYM+I+G
GAPDH	534	217	197	0.22	0.26	0.26	0.26	GTR+I+G	GTR+I+G
NaK 1st codon	204	63	38	0.25	0.19	0.37	0.19	GTR+I+G	GTR+I+G
NaK 2nd codon	204	35	14	0.36	0.21	0.17	0.26	TVM+I+G	GTR+I+G
NaK 3rd codon	204	194	180	0.13	0.35	0.28	0.24	GTR+I+G	GTR+I+G
NaK 1st +2nd codons	408	98	52	0.30	0.20	0.27	0.23	TrN+I+G	GTR+I+G
NaK	612	292	232	0.25	0.25	0.28	0.23	GTR+I+G	GTR+I+G
PEPCK 1st codon	183	60	45	0.27	0.20	0.36	0.17	GTR+I+G	GTR+I+G
PEPCK 2nd codon	183	33	24	0.26	0.30	0.21	0.24	SYM+I+G	SYM+I+G
PEPCK 3rd codon	183	167	164	0.15	0.36	0.25	0.24	GTR+I+G	GTR+I+G
PEPCK 1st +2nd codons	366	93	69	0.26	0.25	0.29	0.20	TVMef+I+G	SYM+I+G
PEPCK	549	260	233	0.23	0.29	0.27	0.22	GTR+I+G	GTR+I+G
All 1st codon	888	222	154	0.27	0.19	0.37	0.17	GTR+I+G	GTR+I+G
All 2nd codon	888	129	80	0.31	0.24	0.18	0.27	GTR+I+G	GTR+I+G
All 3rd codon	888	814	768	0.10	0.38	0.26	0.26	GTR+I+G	GTR+I+G
All data	2664	1165	1002	0.23	0.27	0.27	0.23	GTR+I+G	GTR+I+G

MP analyses together on the inferred Bayesian topology (Fig. 2).

Phylogenetic Relationships among Anomurans

Anomura is strongly supported as monophyletic. As currently conceived, Paguroidea and Galatheoidea are polyphyletic. Of the three anomuran superfamilies with more than one family, only the Hippoidea are monophyletic. Chirostylidae, Diogenidae, and Paguridae are each paraphyletic with the incursion of Kiwaidae, Coenobitidae, and Lithodidae, respectively. The placement of king crabs (Lithodidae) within the asymmetrical hermit crab clade, Paguridae, is consistent with previous molecular analyses (Cunningham et al. 1992; Morrison et al. 2002; Tsang et al. 2008; Ahyong et al. 2009), though not with the conclusions of McLaughlin and Lemaitre (1997) and McLaughlin et al. (2007) based on adult morphology and with McLaughlin et al. (2004) based on larval morphology. Note however that results of McLaughlin et al. (2004) are either inconclusive (their figure 7) or actually show lithodids to be nested within the Paguridae (their figure 6). Pylochelidae is polyphyletic such that the two subfamilies analyzed, Trizochelinae (represented by *Trizocheles*) and Pylochelinae (represented by *Pylocheles* and *Xylocheles*) are widely dispersed. Monophyly of Pylochelidae is strongly rejected by the SH test ($P = 0.007$) and BF (63.9). Other paguroid clades are widely dispersed and an a priori hypothesis of a hermit crab clade is significantly worse than the inferred phylogeny, irrespective of whether Lithodoidea (king crabs) are included in Paguroidea (SH test, $P < 0.001$, BF = 147.5) or excluded (SH test, $P < 0.001$, BF = 1261). Similarly, an alternative hypothesis of Galatheoidea monophyly is also rejected (SH test, $P < 0.001$, BF = 199.8). Galatheid squat lobsters and porcelain crabs (Porcellanidae) are most closely related to the symmetrical hermit crab clade Pylochelinae. The other squat lobsters (Chirostylidae, Kiwaidae, and Aegloidae) and hairy stone crab (Lomisidae) are allied to the asymmetrical hermit crab clade, Parapaguridae, and the symmetrical hermit crab lineage, Trizochelinae.

We inferred the ancestral body form of the most recent common ancestor (MRCA) of different lineages based on the Bayesian consensus topology (Fig. 4). The likelihood of different ancestral states is indicated by the pie chart on the corresponding nodes. The ancestral state reconstructions suggest that the hermit crabs had a single origin, during the divergence between Hippoidea and the MRCA of the remaining anomurans, and that other

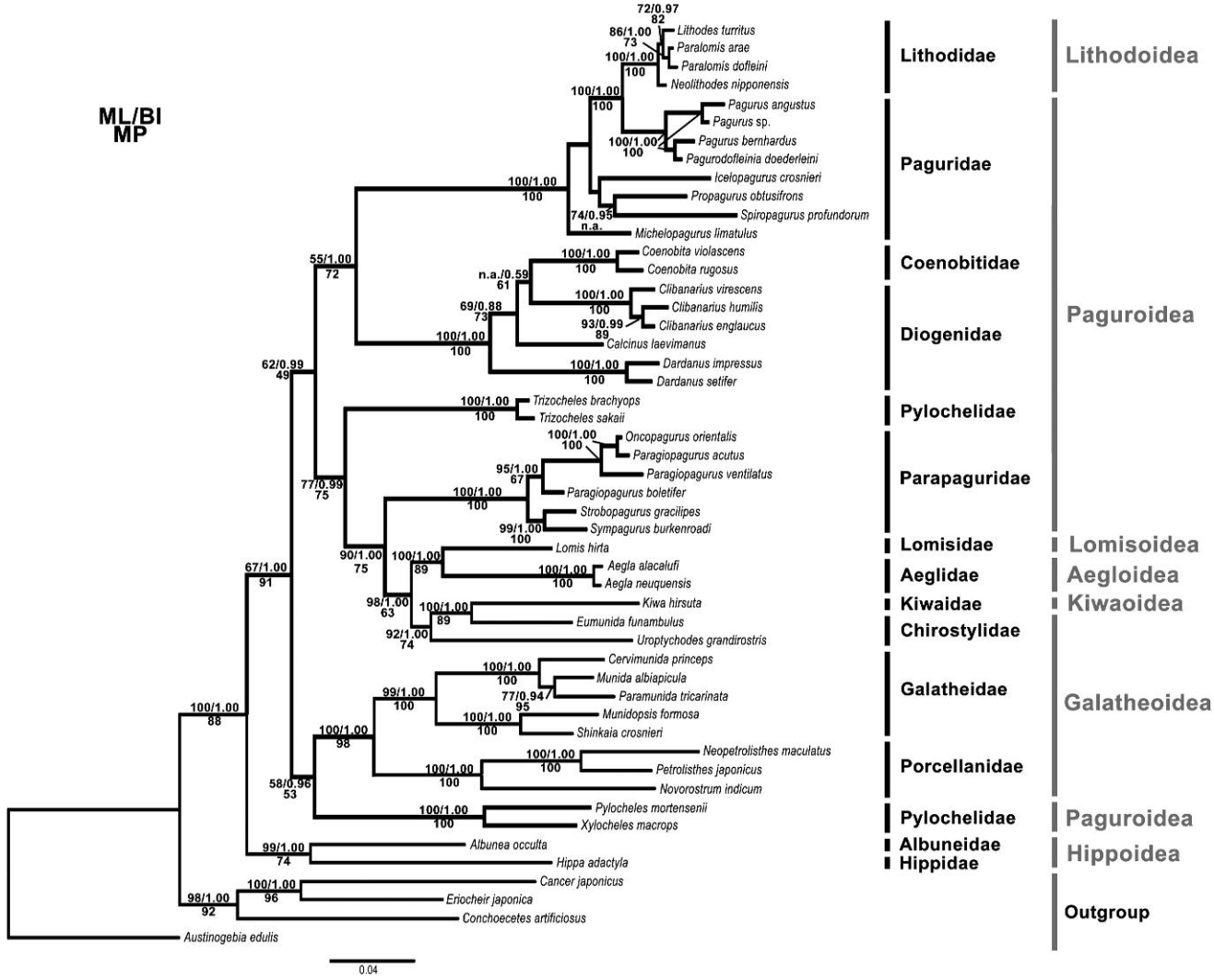

FIGURE 2. Bayesian consensus topology of the combined five-gene data set partitioned by gene. Nodal supports are denoted on the corresponding branches and values under 50 (for MP and ML) or 0.5 (for BI) are represented by "n.a.". The superfamily and family classifications are denoted by the grey and black bars at the right, respectively.

major body forms were derived from the symmetrical hermit crabs. Coincidently, both the two crab-like forms, porcellanids and lomisids, appear to have evolved independently from symmetrical hermit ancestors, via a squat lobster–like intermediate (Fig. 4). The king crab is the only crab-like animal derived from an asymmetrical hermit-like ancestor. The two alternative topologies (B and C in Fig. 3) inferred from other partition schemes also support a single origin of hermit crabs, and double transition from symmetrical hermit crab to squat lobster and finally converging on a crab-like form (data not shown).

DISCUSSION

Comparison to Previous Molecular Phylogenetic Hypotheses

Various attempts have been made to reconstruct the anomuran phylogeny based on molecular data (Morrison et al. 2002; Pérez-Losada et al. 2002; Ahyong and O'Meally 2004; Tsang et al. 2008; Ahyong et al. 2009). The two major studies by Morrison et al. (2002) and Ahyong et al. (2009) are, however, split in their conclusions. One of the major disagreements in their phylogenetic hypotheses is the monophyly of the hermit crabs. Morrison et al. (2002), using mitochondrial gene rearrangements, recovered a single origin of hermit crabs. This was supported by a translocation of the *Leu*$_{(CUN)}$ tRNA gene from a position between *COI* and *COII* to the position next to *Leu*$_{(UUR)}$ (their mitochondrial rearrangement 3). This was followed by another rearrangement of the *Leu*$_{(UUR)}$ tRNA to the middle of *COI* and *COII* (their mtDNA rearrangement 4). The former has aligned the Hippoidea as the sister clade to hermit crabs, whereas the latter defines a single origin of all hermits (Morrison et al. 2002). As a result, the Galatheoidea is placed at the base. On the contrary, based on the analysis of mitochondrial 16S and nuclear

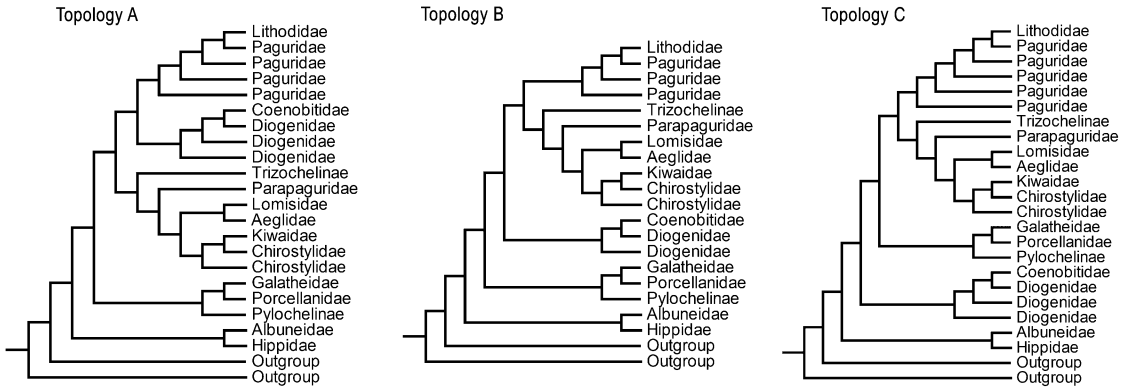

FIGURE 3. The three topologies resulting from the phylogenetic analyses under different partition schemes in the present study. Only the family/subfamily names are presented in the diagram for ease of comparison. Please refer to Supplementary Material for the detailed phylogeny and nodal supports.

18S and 28S rDNA sequences, Ahyong et al. (2009) argue for a polyphyly of hermit crabs with the asymmetrical hermit crabs independently evolving two or possibly three times (in Paguridae, Parapaguridae, and Diogenidae + Coenobitidae). Moreover, they propose the Hippoidea as the basal anomurans (Ahyong et al. 2009). We find strong support for the basal position of Hippoidea, consistent with the hypothesis of Ahyong et al. (2009). However, our gene tree suggests the asymmetrical hermit crab abdomen has evolved only twice, in the Parapaguridae and the common ancestor of other asymmetrical hermits (i.e., Paguridae, Diogenidae, and Coenobitidae) instead of three as suggested by some of the rDNA sequence-based results (Ahyong et al. 2009). Statistical support at several of the critical nodes of the previous rDNA phylogeny is low, however, so disagreements between our present topology and the rDNA phylogeny may be more superficial than actual.

On the other hand, given the polyphyly of the hermit crabs, the mitochondrial gene rearrangements discovered by Morrison et al. (2002) do not represent synapomorphies but have arisen multiple times within the Anomura. The "rearrangement" of the leucine tRNA gene could occur through a process of tRNA duplication and mutation in the anticodon triplet (Rawlings et al. 2003). The newly remolded leucine tRNA genes subsequently take over the isoaccepting L_{CUN} leucine tRNA, resulting in the apparently rearranged mtDNA (Rawlings et al. 2003). This process has been shown to have occurred at least seven times in animal evolution. The leucine tRNA gene sequences of the taxa included in the Morrison et al. (2002) study exhibit a strong signal of a putatively recent remolding event as suggested by the high similarity in the sequence of the two different leucine tRNA genes from the same species (Rawlings et al. 2003). Therefore, we suggest that the mtDNA "rearrangements" that occurred in the anomurans are the products of multiple tRNA duplication and mutation events rather than rearrangements. The nodal support of the phylogeny of Morrison et al. (2002) is weak and inconclusive without the topological constraint derived from the mtDNA rearrangements. In sum, our topology inferred from multiple independent nuclear protein-coding gene loci is substantially more robust than any of the previous molecular phylogenetic hypotheses proposed to date.

Evolution of Hermit Crabs, Crab-like and Squat Lobster Forms

Our results not only document significant polyphyly among anomuran taxa that were long thought to be monophyletic, they also show parallel evolution of several markedly different types of body forms in the Anomura including plausible transitional trends toward carcinization. Moreover, each of these body forms has been derived from within clades of hermit crabs (whether symmetrical or asymmetrical). As expected, the crab-like form is achieved via a progressive broadening of the cephalothorax and shortening of the pleon, which is held partially "tucked under," followed by a further significant reduction of the pleon, which is held fully folded under the cephalothorax. The transition proceeds from the long-tailed symmetrical hermit crab through the squat lobster form or asymmetrical hermit crab form, and finally to crab-like form.

The earliest fossil attributed to Anomura dates to the Upper Triassic (Chablais et al. 2011); it is a symmetrical, long-tailed somewhat 'lobster-like' form and its affinities require further study. Nevertheless, the fossil record indicates that the paguroids emerged early in anomuran evolution, diverging from the sister clade, Hippoidea (Pérez-Losada et al. 2002; Ahyong and O'Meally 2004; Porter et al. 2005; Tsang et al. 2008; Ahyong et al. 2009) by at least the early Jurassic, as evidenced by isolated fossil chelae of indeterminate familial placement. More complete hermit crab fossils, including some attributed to the symmetrical Pylochelidae, are known from the Late Jurassic onward (van Bakel et al. 2008). Galatheid squat lobsters and porcellanids are known from the Middle Jurassic, chirostylids and aeglids from the Cretaceous (Feldmann et al. 1998; Schweitzer and Feldmann

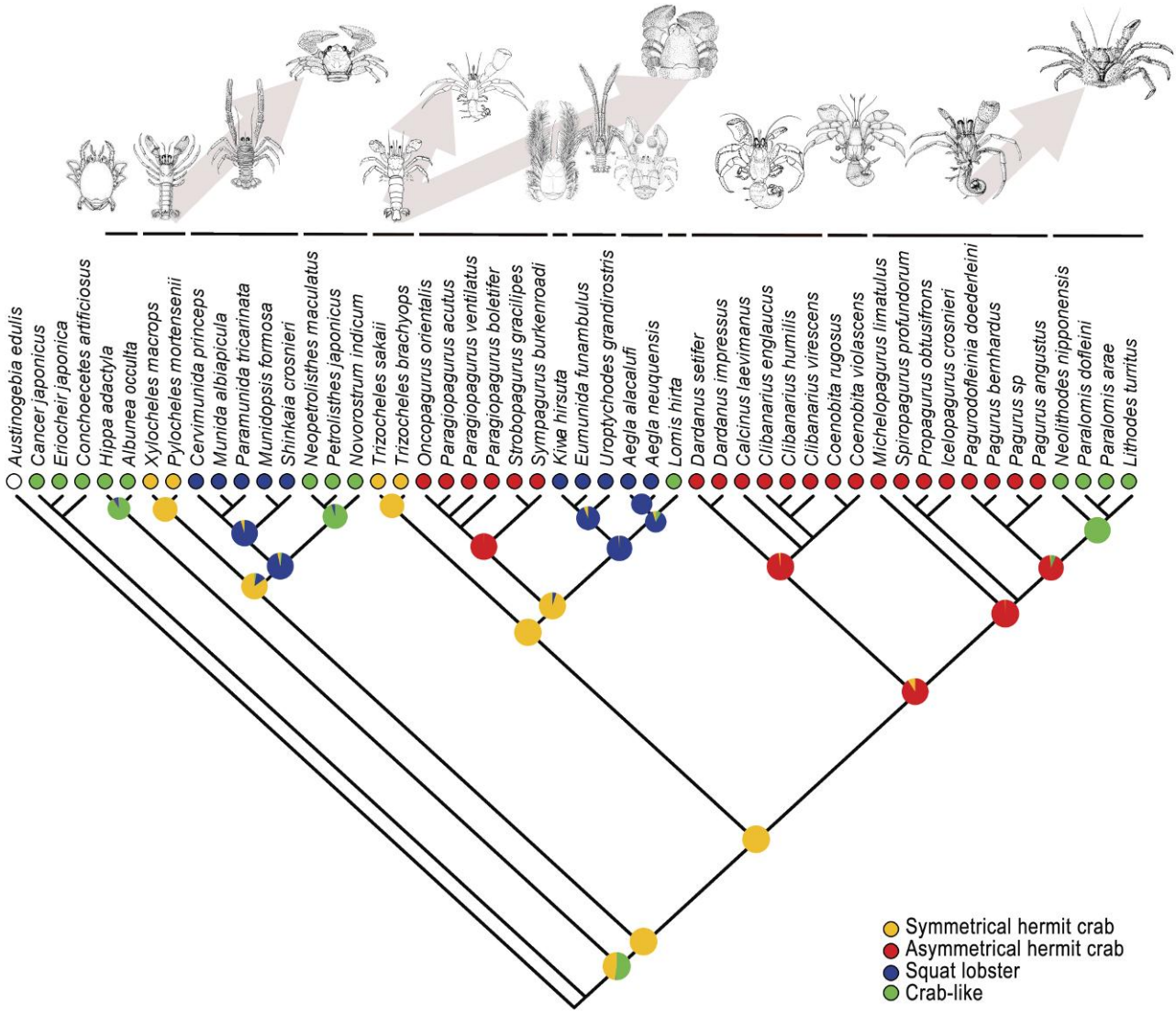

FIGURE 4. Maximum likelihood ancestral state reconstruction of the different body forms of the anomuran lineages based on the Bayesian consensus tree (partition by gene). The relative likelihoods of alternative ancestral states are indicated by pie charts on the corresponding nodes. The circles below the species names indicate the body form of the species and the bars above encompass members of the same family. The sketches are representatives of the corresponding families but may not illustrate the species examined in the present study. The arrows indicate the inferred direction of evolution in body form among the families.

2000, 2010), and lithodids from the Miocene (Feldmann et al. 1998). Thus, our results are consistent with these fossil findings, though we have not attempted to estimate the timing of hermit crab radiations. Most other major anomuran clades were derived from symmetrical hermit crab ancestors. Additionally, the basal positions of the pylochelid clades indicate that pylochelid symmetry is plesiomorphic, rather than secondarily acquired. Derivation of squat lobsters from symmetrical hermit crab ancestors is also consistent with fossil evidence. For instance, the fossil galatheid squat lobster, *Munitheites*, possesses morphological features in common with early symmetrical hermit crabs, indicating possible shared ancestry (van Bakel et al. 2008).

The *Lomis* + *Aegla* clade has been recovered by previous molecular studies (Morrison et al. 2002; Ahyong and O'Meally 2004; Ahyong et al. 2009), though their nearest relatives have long been enigmatic; both have been variously posited as relatives of hermit crabs (and squat lobsters in the case of *Aegla*) (Martin and Abele 1986; Pérez-Losada et al. 2002). *Aegla* resembles galatheid and especially kiwaid squat lobsters in overall body form, but its pleon is proportionally shorter and can be considered to be more highly carcinized than its marine counterparts (Fig. 1q). *Lomis*, the sister to *Aegla*, is strongly crab-like and is highly carcinized (Fig. 1t). Thus, a carcinization trend is fully consistent with a transition from chirostylid/kiwaid and aeglid to lomisid. The common ancestor of *Lomis* and *Aegla* probably had a much reduced pleon compared with the more elongated form observed in modern Chirostylidae and Kiwaidae. Unlike most squat lobsters, which

are free-living or coral associates, *Lomis* and *Aegla* live under boulders and stones, the former on intertidal rocky shores of southern Australia and the latter in flowing freshwater creeks and streams of South America. For both animals, a short compact pleon is probably advantageous in exploiting crevices in rocky habitats as shown in other sympatric brachyuran crabs. Concordantly, the porcelain crabs, which are also derived from long-tailed galatheid ancestors, are predominantly shallow water inhabitants that also usually exploit similar habitats as *Lomis*. They may have experienced similar selective pressures as *Lomis* and *Aegla*, resulting in parallel carcinization. This phenomenon is consistent with the Morrison et al. (2002) hypothesis of a shallow water origin of carcinization. The multiple independent circumstances of transition offer strong evidence for the adaptive advantages of carcinization in relation to habitat type.

The king crabs (Lithodidae) are the only crab-like anomurans to be derived from asymmetrical hermit crabs (Paguridae). The porcellanids and lomisids, both of which are derived from symmetrical ancestors, retain the symmetrical pleon. Likewise, the king crabs appear to display clear traces of pagurid ancestry in pleonal and cheliped asymmetry. McLaughlin et al. (2004) argued that the pleonal asymmetry of lithodoids and paguroids is not homologous because the developmental stages are not directly parallel, and the right-handedness shared by both groups is not necessarily homologous. However, the deeply nested position of king crabs within the asymmetrical hermit crabs strongly suggests that pleon asymmetry has homologous origins even if its precise ontogenetic expression is no longer identical to that of the common ancestor. Similarly, the phylogenetic position of the lithodoids within pagurids indicates that right-handedness is homologous. Lithodoids differ from most paguroids in having sexually dimorphic pleonal asymmetry—symmetrical in males and asymmetrical in females. It is noteworthy then that one of the partially "carcinized" parapagurid hermit crabs, *Probeebei*, also exhibits sexually dimorphic pleonal asymmetry (Wolff 1961).

Prevalence of Parallel Evolution in Anomura

Our phylogenetic results demonstrate that the deep sea asymmetrical hermit crab clade, Parapaguridae, is not closely related to the Paguridae or other asymmetrical hermit crabs but is closer to squat lobsters (chirostylids, kiwaids, aeglids) and crab-like lomisoids. This indicates that pleonal asymmetry and decalcification evolved independently in two different lineages, presumably to exploit ammonite shells or coiled gastropod shell habitats. Additionally, such a finding is consistent with the carcinized morphology of some very rare parapagurids, *Tylaspis* and *Probeebei*. The tendency toward acquisition of crab-like form is widespread throughout Anomura.

The squat lobster body form, exhibited by Galatheidae, Chirostylidae, Kiwaidae, and Aeglidae (all formerly grouped together under Galatheoidea), has evolved independently at least twice: once in the common ancestor of Chirostylidae, Kiwaidae, and Aeglidae + Lomisidae, and once in Galatheidae. Out of these "squat lobster" clades, two independent carcinization events have occurred: one in the porcelain crabs (Porcellanidae), which are sister to Galatheidae, and one in Lomisidae, sister to Aeglidae. The squat lobster form can be plausibly regarded as an intermediate morphology, a case of partial carcinization through the widened cephalothorax and sternal plate (in comparison with hermit crabs), and pleonal disposition, which although well developed, is always carried folded and partially concealed by the cephalothorax (Figs. 1n–q and 5). Thus, in each case of carcinization, a transition pathway from long-tail (i.e., Pylochelidae) to squat lobster to crab-like form (Figs. 4 and 5) is consistent with the phylogeny. On the other hand, the king crab is the only crab-like anomuran derived from asymmetrical hermit crabs (Paguridae) (Figs. 4 and 5). In contrast to the modification of symmetrical forms, asymmetrical pleonal reduction is associated with a shift from linear

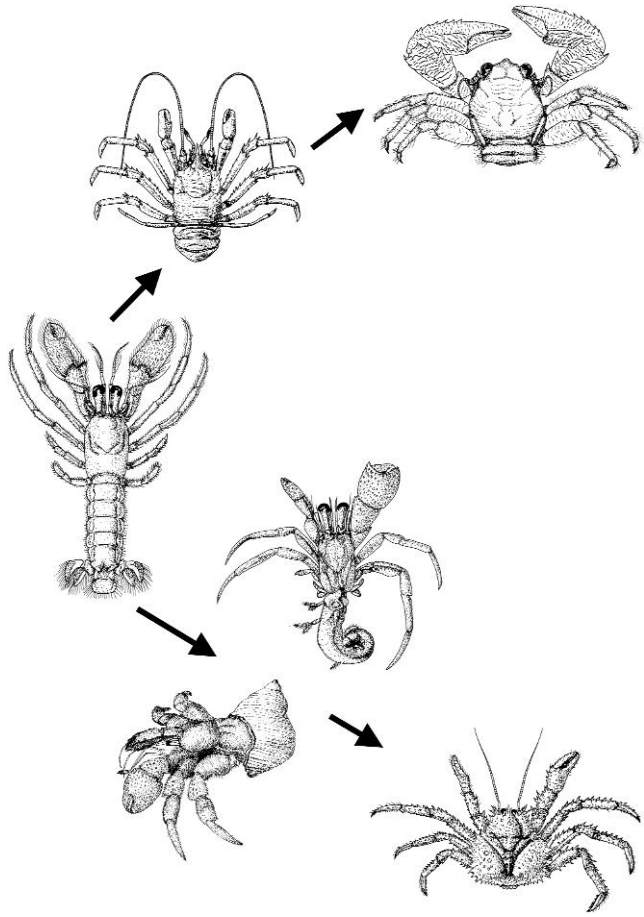

FIGURE 5. Schematic representation of stages of carcinization starting from a symmetrical hermit crab form (Pylochelidae) leading to crab-like forms via different pathways. Upper path, via squat lobster form (e.g., Galatheidae) to crab-like form (e.g., Porcellanidae). Lower path, via asymmetrical hermit crab form (e.g., Diogenidae, Paguridae) to crab-like form (e.g., Lithodidae).

to dextrally coiled carcinoecia, independently derived in Parapaguridae and remaining asymmetrical hermit crabs. The asymmetrical hermit crabs can also be considered to be partially carcinized, having undergone partial pleonal reduction. Carcinization pathways of the king crabs are thus similar to those of symmetrically carcinized forms—a long-tailed plesiomorphic form followed by an intermediate form (pleonal reduction via adaptation to dextral shell carrying), culminating in the crab-like form (Lithodidae). Just as the other two crab-like anomurans (Porcellanidae and Lomisidae) have symmetrical pleons, derived from symmetrical ancestors, respectively, the king crabs display clear traces of pleonal asymmetry consistent with their pagurid ancestry.

Some authors have concluded that the hermit to king hypothesis is developmentally infeasible, as it would require reversal in morphology of complex characters related to dextral shell habitation, and this requires the maladaptive scenario of an asymmetrical shell carrier to abandon the gastropod shell to expose its soft abdomen (McLaughlin and Lemaitre 1997; McLaughlin et al. 2004). Yet, the crab-like terrestrial coconut crab *Birgus latro* (family Coenobitidae) (Fig. 1f), whose nearest relatives all use gastropod shell shelters, is a good example demonstrating ontogenetic carcinization. Juvenile coconut crabs have a soft pleon like most other asymmetrical hermit crabs and reside in a gastropod shell for protection (Reese 1968). With increasing size, the body becomes more robust and crab-like. Adult coconut crabs are free living without dependence on a gastropod shell. Thus, within the ontogeny of a contemporary species, abandonment of the gastropod shell is already demonstrable. Furthermore, larval studies of the asymmetrical hermit crab *Clibanarius vittatus* reveal that the asymmetry is partially influenced by environment (Harvey 1998). Juveniles are asymmetrical, but in the absence of a gastropod shell, the initial asymmetry is weakened and pleonal calcification increased. The degree of pleonal asymmetry and calcification in hermit crabs is environmentally mediated (Harvey 1998). A number of non-gastropod shell living hermit crabs of the families Paguridae (Fig. 1g), Diogenidae (Fig. 1h), and even Parapaguridae (Fig. 1i) that occupy crevices in coral, rock or worm tubes, and bivalve and tusk shells have a symmetrical, though non-calcified, pleon. Given that pleonal asymmetry has independent derivations within the Anomura, it is reasonable to anticipate that most, if not all, of the anomurans have retained the genetic potential for significant changes in body plan.

The independent cases of carcinization in Anomura, each with a similar possible transition series, are products of parallel evolution. This raises the question of the nature of developmental constraint in hermit crabs and allies that lead to the remarkable prevalence of parallel evolution within the group. A major question concerning the phylogenetic separation of these superficially similar groups of Anomura is whether they have arisen from convergence of different developmental pathways or through genetically homologous parallelism. Either way, body form transition is much more evolutionarily plausible than previously thought for Anomura, resulting in repeated derivation of various crab-like and squat lobster forms as well as asymmetrical forms.

The parallel derivation of multiple anomuran body types from the hermit crabs helps account for past controversies over the sister relationships of major groups. Most non-paguroid families have, with good morphological evidence, been variously posited as sister to the hermit crabs. Little wonder that anomuran phylogeny is so contentious. Paradoxically, these contradictory hypotheses are now simultaneously plausible. With the recognition that the major anomuran body forms arose from within the paguroids, it is evident that all major groups of anomurans are indeed closely related to hermit crabs, albeit different clades of hermit crabs. Thus, rather than hermit to king, evolution within the paguroids may be more aptly described as "hermit to all," that is, to "squatters and kings."

Systematic Implications

The long-standing high-level classification of Anomura dominated by Galatheoidea, Paguroidea, and Hippoidea has been largely based on superficially similar body forms, though McLaughlin et al. (2007) provided further refinements. The extensive degree of parallelism in anomuran body forms, however, considerably destabilizes the current classification, chiefly the Paguroidea and Galatheoidea, neither of which are monophyletic as currently conceived. Our results indicate that the classification of the Anomura requires significant revision if it is to reflect phylogenetic relationships.

Conclusions

Our molecular data have explicitly revealed for the first time the parallel evolution of both the squat lobster and crab-like forms from symmetrical hermit crab ancestors. Moreover, the asymmetry in hermit crabs, associated with dextral gastropod shell habitation has independently evolved at least twice and like the squat lobster form, can be considered to represent partial carcinization. We are not aware of any similar cases within the Crustacea of similar flexibility in body form transformation. The multiple and independent parallel evolution of various similar body forms in Anomura not only suggests unexpected evolutionary flexibility and conservation of developmental mechanisms within the group but also raises important questions about how similar selective pressures might induce parallelism in distantly related taxa. This stands in stark contrast to the sister group to the anomurans, the true crabs (Brachyura), which exhibit 4-fold greater species richness and 6-fold greater generic richness but with an essentially uniform body plan. This suggests that Anomura retains a higher degree of inherent evolutionary flexibility than true crabs, which have become canalized along a narrower albeit exceptionally successful evolutionary trajectory. The contribution of intrinsic

and extrinsic factors to morphological evolution has been widely debated, but comparison of Anomura and Brachyura suggests that the greater disparity we see in Anomura may more closely reflect intrinsic factors.

Supplementary Material

Supplementary material, including data files and/or online-only appendices, can be found at http://www.sysbio.oxfordjournals.org/.

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Notes added in proof

Since this paper went to press, two studies revising the classification of the squat lobsters have been published (Ahyong et al., 2010; Schnabel and Ahyong, 2010), which formally recognize Galatheoidea and Chirostyloidea for clades identified herein. Galatheoidea is restricted to the least inclusive clade in Fig. 2 that spans *Novorostrum and Paramunida*. Galatheoidea is divided into four families, Galatheidae, Munididae, Munidopsidae and Porcellanidae, of which the latter three families correspond to the galatheoid clades recovered in Fig. 2 that contain *Cervimunida, Munidopsis* and *Novorostrum*, respectively. The clade in Fig. 2 that comprises *Uroptychus*, *Eumunida* and *Kiwa*, is now known as Chirostyloidea. Chirostyloidea contains Chirostylidae, Eumunididae, Kiwaidae, corresponding in Fig. 2 to *Uroptychodes*, *Eumunida* and *Kiwa*, respectively. Ahyong, S.T., Baba, K., Macpherson, E., Poore, G.C.B. 2010. A new classification of the Galatheoidea (Crustacea: Decapoda: Anomura). Zootaxa, 2676: 57–68. Schnabel, K.E., Ahyong, S.T. 2010. A new classification of the Chirostyloidea (Crustacea: Decapoda: Anomura). Zootaxa, 2687: 56–64.

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