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Galatheoidea are not monophyletic – Molecular and morphological phylogeny of the squat lobsters (Decapoda: Anomura) with recognition of a new superfamily

K.E. Schnabel^{a,*}, S.T. Ah Yong^b, E.W. Maas^a^a National Institute of Water and Atmospheric Research, Private Bag 14 901, Kilbirnie, Wellington, New Zealand^b Australian Museum, 6 College St., Sydney NSW 2010, Australia

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ABSTRACT

The monophyletic status of the squat lobster superfamily Galatheoidea has come under increasing doubt by studies using evidence as diverse as larval and adult somatic morphology, sperm ultrastructure, and molecular data. Here we synthesize phylogenetic data from these diverse strands, with the addition of new molecular and morphological data to examine the phylogeny of the squat lobsters and assess the status of the Galatheoidea. A total of 64 species from 16 of the 17 currently recognised anomuran families are included. Results support previous work pointing towards polyphyly in the superfamily Galatheoidea and Paguroidea, specifically, suggesting independent origins of the Galatheidae + Porcellanidae and the Chirostylidae + Kiwaidae. Morphological characters are selected that support clades resolved in the combined analysis and the taxonomic status of Galatheoidea *sensu lato* is revised. Results indicate that Chirostylidae are more closely related to an assemblage including Aegloidea, Lomisoidea and Paguroidea than to the remaining Galatheoidea and are referred to the superfamily Chirostyloidea to include the Chirostylidae and Kiwaidae. A considerable amount of research highlighting morphological differences supporting this split is discussed. The Galatheoidea *sensu stricto* is restricted to the families Galatheidae and Porcellanidae, and diagnoses for both Chirostyloidea and Galatheoidea are provided. Present results highlight the need for a detailed revision of a number of taxa, challenge some currently used morphological synapomorphies, and emphasise the need for integrated studies with wide taxon sampling and multiple data sources to resolve complex phylogenetic questions.

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1. Introduction

Crustaceans of the suborder Anomura MacLeay, 1838 have received considerable attention in the last decade with major taxonomic and systematic revisions in the superfamilies Hippoidea (Boyko, 2002), Paguroidea (McLaughlin, 2003; McLaughlin et al., 2007) and Galatheoidea (Baba, 2005; Baba et al., 2008; Pérez-Losada et al., 2002; Macpherson, 2007) with the description of many new taxa including new families: Blepharipodidae in the hippoids (Boyko, 2002), Pylojacquesidae in the paguroids (McLaughlin and Lemaitre, 2001) and Kiwaidae in the galatheoids (Macpherson et al., 2005). Anomura presently include 17 families, 223 genera and approximately 2500 species, of which the superfamily Galatheoidea comprises the largest group. They represent a morphologically and ecologically diverse taxon that includes 69 genera (31%) with 1200 species worldwide and they are a prominent element of habitats ranging from shallow-water coral reefs to deep-sea

hydrothermal vents (De Grave et al., 2009; Baba et al. 2008; Osawa, 2007). Galatheoidea currently contains the Chirostylidae, Galatheidae (squat lobsters) and Porcellanidae (porcelain crabs) at last revision (McLaughlin et al., 2007). The common name 'squat lobster' is also used for the freshwater Aeglididae and the 'yeti crab' *Kiwa hirsuta*, described by Macpherson et al. (2005) from deep-sea vents. Both of these taxa were only recently excluded from the Galatheoidea by McLaughlin et al. (2007) and placed into the Aegloidea and Kiwaoidea, respectively.

Galatheoidea have been very weakly represented in phylogenetic studies of the Anomura. In several recent phylogenetic studies the sample size of galatheids, chirostylids and porcellanids combined ranges from two (Dixon et al., 2003; Porter et al., 2005; Tsang et al., 2008) to seven taxa (Chu et al., 2009). Ah Yong et al. (2009) included 19 known genera of galatheids, but analysed only molecular characters. Apart from the position of the Aeglididae, the monophyly of the Galatheoidea has rarely been questioned, despite the fact that it is difficult to find morphological synapomorphies (Ah Yong et al., 2009). Recent studies of adult and larval, somatic and sperm morphology indicate that chirostylids may be more closely related to paguroids than other galatheids (Clark and Ng, 2008; Guerao et al., 2006; Jamieson and Tudge,

* Corresponding author. Address: National Institute of Water and Atmospheric Research, 302 Evans Bay Parade, Greta Point, Private Bag 14 901, Kilbirnie, Wellington, New Zealand. Fax: +64 4 386 2153.

E-mail address: k.schnabel@niwa.co.nz (K.E. Schnabel).

2000) and recent phylogenetic studies support the paraphyly of galatheids and chirostylids (Ahyong et al., 2009; Bracken et al., 2009; Chu et al., 2009). As a consequence, the hypothesis that Galatheaidea is monophyletic is now significantly challenged. However, these studies remain independent and have not resulted in classificatory changes.

This study combines previously published and new DNA sequence data for three ribosomal genes (mitochondrial 16S rRNA and nuclear 18S and 28S rRNA) with 125 morphological characters that include a range of adult and larval somatic and sperm morphology. The combined dataset contains 52 anomuran genera in 16 of the 17 families and 30 of the 68 known galatheid genera and represents the synthesis of molecular and morphological data. It aims to consolidate recent hypotheses into a single framework addressing commonalities and discrepancies in the ongoing and vigorous debate over internal anomuran relationships with a specific focus on the status and position of Galatheaidea. Within this context, the question to be addressed here is whether a monophyletic taxon Galatheaidea can be validated.

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 Galatheaidea (Table 1). All three galatheid subfamilies were included; representing 18 of 34 recognised genera. Porcellanidae was represented by five genera (of 28) and Chirostylidae was represented by all six of the seven accepted genera.

Table 1
Classification of terminal taxa with GenBank accession numbers for gene sequences. *Shinkaia* 16S and 18S sequence provided by K. H. Chu (CUHK, Chinese University of Hong Kong). The high-level classification follows McLaughlin et al. (2007). Reference to samples previously published by Ahyong and O'Meally (2004): 'A and O (2004)' and Ahyong et al. (2009): 'A. et al.'. Species or congeners scored *de novo* for adult morphology characters are indicated by a '*'. 'X' indicates missing data.

TAXON		16S	18S	28S	Reference
ANOMURA					
HIPPOIDEA					
Blepharipodidae	<i>Blepharipoda occidentalis</i>	AF436053	AF436014	AF435994	A and O (2004)
Hippidae	<i>Emerita emeritus</i>	AY583898	AY583971	AY583990	A and O (2004)
Albunidae	<i>Lepidopa californica</i>	AF436054	AF436015	AF435996	A and O (2004)
PAGUROIDEA					
Coenobitidae	<i>Coenobita compressus</i>	*	AF436059	AF436023	AF435999
Coenobitidae	<i>Birgus latro</i>	*	HQ380270	HQ380295	HQ380282
Diogenidae	<i>Calcinus obscurus</i>	*	AF436058	AF436022	AF435998
Diogenidae	<i>Clibanarius albidigitus</i>		AF425323	AF438751	AF425342
Diogenidae	<i>Isocheles pilosus</i>		AF436057	AF436021	X
Diogenidae	<i>Paguristes1 turgidus</i>		AF436056	AF436020	AF435997
Diogenidae	<i>Paguristes2 pilosus</i>	*	HQ380271	HQ380296	HQ380283
Paguridae	<i>Bythiopagurus macrocolus</i>	*	EU821532	EU821548	EU821565
Paguridae	<i>Discorsopagurus schmitti</i>		AF436055	AF436017	X
Paguridae	<i>Pagurus spp.</i>	*	AF425335	AF436018	AF425354
Paguridae	<i>Porcellanopagurus filholi</i>	*	HQ380267	HQ380291	HQ380278
Parapaguridae	<i>Parapagurus latimanus</i>	*	EU821534	EU821550	EU821567
Parapaguridae	<i>Sympagurus dimorphus</i>	*	EU821533	EU821551	EU821568
Pylochelidae	<i>Pylocheles macrops</i>	*	AY583897	AY583970	AY583989
Pylochelidae	<i>Trizocheles spinosus</i>	*	EU821535	EU821551	EU821568
LITHODOIDEA					
Lithodidae	<i>Lithodes santolla</i>	*	AY595927	AF439385	AY596100
Lithodidae	<i>Oedignathus inermis</i>		AF425334	Z14062	AF425353
LOMISOIDEA					
Lomisidae	<i>Lomis hirta</i>	*	AF436052	AF436013	AF435993
AEGLOIDEA					
Aeglididae	<i>Aegla uruguayana</i>	*	AF436051	AF436012	AF435992
Aeglididae	<i>Aegla violacea</i>	*	AY595880	AY595799	AY596051
KIWAOIDEA					
Kiwaidae	<i>Kiwa hirsuta</i>	*	EU831284	DQ219316	EU831286

Tissue samples were derived from specimens or tissue loaned from the collections of the Natural History Museum of Los Angeles County (LACM), Natural History Museum of Vienna (NHMW), Muséum national d'Histoire naturelle, Paris (MNHN), National Taiwan Ocean University, Keelung, Taiwan (NTOU), and the National Institute of Water and Atmospheric Research, Wellington, New Zealand (NIWA). The analysis was rooted to two basal brachyurans, *Lauridromia dehaani* and *Paromola japonica*, based on inferred sister relationships from other studies. The majority of phylogenetic analyses of high-level decapod interrelationships (e.g., Scholtz and Richter, 1995; Dixon et al., 2003; Ahyong and O'Meally, 2004; Tsang et al., 2008) have recovered a Brachyura as sister to Anomura, as Scholtz and Richter's (1995) clade, Meiura. Some recent studies have not recovered a Meiura clade (e.g., Porter et al., 2005; Bracken et al., 2009), but the nodal support for the positions of Brachyura and Anomura was low. Thus, at present, the best corroborated sister group to Anomura is Brachyura.

2.2. Molecular data

Two nuclear ribosomal genes (nearly complete 18S rRNA, and the D1 region of 28S rRNA) and one partial mitochondrial ribosomal gene (16S rRNA), were selected for their utility in resolving phylogenetic history at different taxonomic levels. Following the principle of 'total evidence' (see Grant and Kluge, 2003 and references therein), the 16S, 18S and 28S sequences and morphological data were analysed simultaneously.

A total of 64 taxa were included, data for 45 taxa were previously published (Ahyong and O'Meally, 2004; Ahyong et al., 2009), three additional sets were derived from GenBank and se-

Table 1 (continued)

TAXON		16S	18S	28S	Reference	
GALATHEOIDEA						
Chirosstylidae	<i>Chirosstylus novaecaledoniae</i>	*	EU821539	EU821555	EU821572	A. et al.
Chirosstylidae	<i>Eumunida sternomaculata</i>	*	AY351260	AF436011	AF435991	A and O (2004)
Chirosstylidae	<i>Gastroptychus2 novaezelandiae</i>	*	EU821538	EU821554	EU821571	A. et al.
Chirosstylidae	<i>Gastroptychus1 rogeri</i>	*	HQ380260	HQ380285	HQ380272	NIWA
Chirosstylidae	<i>Pseudomunida fragilis</i>	*	EU821536	EU821552	EU821569	A. et al.
Chirosstylidae	<i>Uroptychodes epigaster</i>	*	X	X	HQ380269	NIWA
Chirosstylidae	<i>Uroptychus1 spirostris</i>	*	EU821537	EU821582	EU821570	A. et al.
Chirosstylidae	<i>Uroptychus2 parvulus</i>	*	AY595926	AF439386	AY596097	Genbank
Chirosstylidae	<i>Uroptychus3 scambus</i>	*	EU831282	EU821553	EU831283	A. et al.
Chirosstylidae	<i>Uroptychus4 nitidus</i>	*	AY595925	AF439387	AY596096	Genbank
Chirosstylidae	<i>Uroptychus5 australis</i>	*	X	HQ380270	X	NIWA
Galatheidae	<i>Agononida1 longipes</i>	*	X	AF439381	X	A and O (2004)
Galatheidae	<i>Agononida2 procera</i>	*	EU821540	EU821556	EU821573	A. et al.
Galatheidae	<i>Alainius crosnieri</i>	*	HQ380263	HQ380287	HQ380275	MNHN
Galatheidae	<i>Allogalatea elegans</i>	*	EU821543	EU821560	EU821577	A. et al.
Galatheidae	<i>Anoplionida inermis</i>	*	HQ380265	HQ380289	HQ380276	MNHN
Galatheidae	<i>Babamunida kanaloa</i>	*	FJ858729	HQ380294	HQ380281	LACM
Galatheidae	<i>Bathymunida ballssi</i>	*	HQ380266	HQ380290	HQ380277	MNHN
Galatheidae	<i>Cervimunida johni</i>	*	EU821546	EU821563	EU821580	A. et al.
Galatheidae	<i>Galacantha rostrata</i>	*	HQ380261	EU821559	EU821576	A. et al.
Galatheidae	<i>Galathea sp.</i>	*	EU821544	EU821561	EU821578	A. et al.
Galatheidae	<i>Heteronida spirostris</i>	*	X	HQ380286	HQ380274	MNHN
Galatheidae	<i>Leiogalatea laevirostris</i>	*	EU821541	EU821557	EU821574	A. et al.
Galatheidae	<i>Munida1 quadrispina</i>	*	AF436050	AF436010	AF435990	A and O (2004)
Galatheidae	<i>Munida2 subrugosa</i>	*	AY050075	AF439382	AY596099	A and O (2004)
Galatheidae	<i>Munidopsis bairdii</i>	*	EU821542	EU821558	EU821575	A. et al.
Galatheidae	<i>Neonida grandis</i>	*	HQ380264	HQ380288	X	MNHN
Galatheidae	<i>Pleuroncodes monodon</i>	*	EU821545	EU821562	EU821579	A. et al.
Galatheidae	<i>Sadayoshia sp.</i>	*	EU821547	EU821564	EU821581	A. et al.
Galatheidae	<i>Shinkania crosnieri</i>	*	X	KHC	EU831285	A. et al.
Galatheidae	<i>Torbenella orbis</i>	*	HQ380268	X	X	MNHN
Porcellanidae	<i>Euceramus sp.</i>	*	HQ380268	HQ380292	HQ380279	NHMMW
Porcellanidae	<i>Pachycheles1 haigae</i>	*	AY050076	AF439389	X	A and O (2004)
Porcellanidae	<i>Pachycheles2 rudis</i>	*	AF260598	AF436008	AF435988	A and O (2004)
Porcellanidae	<i>Petrolisthes1 armatus</i>	*	AF436049	AF436009	AF435989	A and O (2004)
Porcellanidae	<i>Petrolisthes2 laevigatus</i>	*	AF260606	AF439388	X	A and O (2004)
Porcellanidae	<i>Porcellana platycheles</i>	*	HQ380269	HQ380293	HQ380280	NIWA
Porcellanidae	<i>Porcellanella triloba</i>	*	EU834069	X	X	A. et al.
BRACHYURA						
DROMIOIDEA						
Dromiidae	<i>Lauridromia dehaani</i>	*	AY583899	AY583972	AY583991	A and O (2004)
HOMOLOIDEA						
Homolidae	<i>Paramola japonica</i>	*	AY583900	AY583973	AY583992	A and O (2004)

quences for 15 taxa are new, 14 of these species have not been included in previous studies (Table 1, Genbank Accession numbers HQ380260–HQ380296). Gene sequences were derived from the same species, except for *Pagurus* where 16S and 28S sequences were from *P. bernhardus* and the 18S sequence from a closely related congener *P. longicarpus*.

2.3. DNA extraction, amplification, sequencing and sequence analysis

Genomic DNA was either directly extracted from fresh or ethanol-fixed tissue samples that were soaked 24 h in a buffer containing 500 mM Tris-HCl (pH 9.0), 20 mM EDTA and 10 mM NaCl. Extraction followed the standard protocol of the QIAGEN DNeasy Blood and Tissue Kit and subsequent quantification of DNA concentration using the Fluorescent DNA Binding Dye PicoGreen™ (Molecular Probes Inc., USA). For problematic taxa, a linear acrylamide precipitation (Ambion, Inc., following protocols recommended by manufacturer) was used overnight to increase concentration of DNA. The entire 18S rRNA gene fragment was amplified using the primer pair 18S-F07 (Medlin et al., 1998) and 18S-R1514 (Sogin, 1990). Subsequently, three internal primers were used for sequencing: 18S-R651, 18S-F551 and 18S-F1053

(Ahyong et al., 2009). A partial sequence of the 16S rRNA gene was amplified using the primer pairs LR-N-13398 and LR-J-12887 (Morrison et al., 2002). This primer proved problematic for some paguroids so an internal primer pair (16S-F85pag and 16S-R492pag) was designed. A partial sequence of the 28S rRNA D1 expansion region was amplified using the primer pair 28S-F216 and 28S-R443 (Ahyong et al., 2009). See Appendix 1 for all primer sequences and first descriptions of the properties of the internal 18S and paguroid 28S primers.

Polymerase chain reactions (PCR) were conducted in 25 µL volumes with 1–5 µL of genomic DNA containing approximately 10 ng of DNA and using primarily Invitrogen Platinum PCR SuperMix containing 22 mM Tris-HCl, 55 mM KCl, 1.65 mM MgCl₂, and 220 µM dNTP. Problematic taxa were sometimes successfully amplified using the lower-fidelity Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare). Conditions for 18S and 28S amplification were an initial denaturation at 94 °C for 2 min, then 30 cycles of 94 °C for 1 min, annealing for 1 min at 50 °C, extension at 72 °C for 2 min and a final extension at 72 °C for 7 min. Conditions for 16S amplification were an initial denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, annealing for 30 s at 50 °C, extension at 72 °C for 90 s and a final extension at 72 °C

for 7 min. PCRs were checked by running 5 μ L of the reaction on a 1% agarose gel stained with Ethidium bromide.

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 the QIAquick Gel Extraction Kit spin columns (Qiagen).

Forward and reverse strands were sequenced using sequencing services of Macrogen Inc., Korea (BigDye™ terminator and ABI Sequencer 3730×, <http://www.macrogen.com>). Forward and reverse sequences were combined and checked for errors using Chromas-Pro Version 1.34 (Technelysium Pty Ltd.) and checked for closest matches in the NCBI Genbank sequence database. Sequences were analysed using the ARB software package version 07.12.07org (Ludwig et al., 2004) and the corresponding SILVA SSU and LSU Ref 95 databases (Pruesse et al., 2007) that provide aligned reference sequences and secondary structure information for more than 45,000 Eukaryota in total. The following reference taxa were used to aid alignment in the respective databases: *Squilla empusa*, *Ibla cumingi*, *Callinectes sapidus*, *Thalassina anomala*, *Syridopsis* sp. and *Arthropoeris* sp. were used in the SSU database and information for *Drosophila melanogaster*, *Protocalliphora azurea* and *Tigriopus californicus* were used as reference in the LSU database. The ARB fast aligner algorithm was used to align all sequences and, subsequently, manual refinement of the alignment was carried out taking into account secondary structure information. The entire DNA sequence dataset contained 3233 nucleotides of which 294 were excluded because they represented large insertions for single taxa (e.g. 100 bp insertion for Porcellanidae in 18S alignment, a 40 bp insertion for *Gastroptychus rogeri* in 28S, and a 52 bp insertion for *Pseudomunida fragilis* in 16S), a total of 2819 nucleotides were contained for final analyses. Sequences containing 2025 base pairs of the nearly complete sequence of 18S, 322 base pairs of the 28S D1 expansion region and 467 base pairs of 16S ribosomal RNA genes were obtained. The alignment is deposited on TreeBASE (<http://www.treebase.org>) (Submission S10907).

2.4. Morphological characters

The morphological data matrix of 125 characters was constructed in MacClade 4.0 (Maddison and Maddison, 2000). The character list and morphological data matrix are shown in Appendix 2 and 3.

Adult morphology: characters were scored from specimens in the NIWA Invertebrate Collection, Australian Museum and published accounts (Table 1). Reviews of the adult somatic morphology were provided by Martin and Abele (1986), Scholtz and Richter (1995) McLaughlin and Lemaitre (1997), Dixon et al. (2003), Ah Yong and O'Meally (2004), and McLaughlin et al. (2007).

Sperm morphology: characters were all extracted from comparative reviews of anomurans by Tudge (1991, 1995, 1997), Tudge et al. (1999), Jamieson and Tudge (2000), Tudge et al. (2001), Tudge and Scheltinga (2002), Tudge (2003). Since sperm characters are phylogenetically conservative, we made assumptions of generic monophyly when scoring sperm characters. Thus, these characters were assumed the same across genera.

Larval morphology: Most consistent information on larval morphology reported on the first zoeal stage larvae of anomurans and only this stage is included. There are very few reviews and comparative studies (Kraus, 2006; MacDonald et al., 1957; McLaughlin et al., 2004; Van Dover et al., 1982), and characters were all scored from following published descriptions: CHIROSTYLIDAE: *Chirostylus ortmanni* (Clark and Ng, 2008), *C. dolichopus* (Ogawa and Matsuzaki, 1992), *Eumunida annulosa* and *E. capillata* (Guerao et al., 2006), *Uroptychus tomentosus* and *Gastroptychus novaeseelandiae* (Pike and Wear, 1969). GALATHEIDAE: *Neonida*

grandis, *Agononida squamosa*, *Babamunida javieri* (Guerao et al., 2006), *A. incerta*, *Munida striola* (Konishi and Saito, 2000), *M. subrugosa* (Roberts, 1973), *M. banffica*, *Galathea strigosa*, *G. dispersa* (Lebour, 1930), *G. squamifera*, *G. intermedia* (Lebour, 1931), *G. intermedia* (Christiansen and Anger, 1990), *Pleuroncodes planipes* (Boyd, 1960), *P. monodon* (Fagetti and Campodónico, 1971), *Sadayoshia edwardsii* (Fujita and Shokita, 2005), *Munidopsis polymorpha* (Wilkins et al., 1990), *Ms. tridentata* (Samuelsen, 1972). PORCELLANIDAE: *Euceramus praelongus* (Roberts, 1968), *Pachycheles serratus* (Rodríguez et al., 2004), *Petrolisthes robsoneae* (García-Guerrero et al., 2005), *Porcellana longicornis*, *P. platycheles* and *Petrolisthes armatus* (Lebour, 1943), *Porcellana cancrisocialis* (García-Guerrero et al., 2006). PAGURIDAE: *Pagurus armatus* (McLaughlin and Gore, 1992). LITHODIDAE: *Lithodes aequispina* (Haynes, 1982), *L. santolla* (Anger et al., 2004). COENOBITIDAE: *Birgus latro* (Reese and Kinzie, 1966), *Coenobita variabilis* (Harvey, 1992), *C. compressus* (Brodie and Harvey, 2001). PARAPAGURIDAE: *Parapagurus diogenes* (Williamson and von Levetzow, 1967). DIOGENIDAE: *Clibanarius aequabilis*, *C. erythropus* (Bartilotti et al., 2008), *Paguristes spinipes* (Provenzano, 1978), *Diogenes miles* (Shenoy and Sankolli, 1993). PYLOCHELIDAE: *Trizocheles spinosus*, *T. vaubanae*, *Pomatocheles jeffreysii*, *Pylocheles mortensenii* (McLaughlin and Lemaitre, 2008), *Pylocheles mortensenii* (Saito and Konishi, 2002). HIPPOIDEA: *Emerita analoga*, *Blepharipoda occidentalis*, *Lepidopa myops* (Johnson and Weldon, 1942), *Lepidopa myops* (Knight, 1970), *Emerita talpoida* (Rees, 1959), hippid larvae (Martin and Ormsby, 1991).

Larval feeding, unless specified, was implied by the presence or absence of setae on the coxae, bases and endopods of the maxillule, maxilla and maxillipeds (Clark and Ng, 2008).

Coding for 97 adult somatic morphological features is based on species used in the molecular analysis. One character [98] was included describing embryonic development as direct (Aeglidae) or indirect (all remaining Anomura) (McLaughlin and Lemaitre, 2008; Scholtz and Richter, 1995). Characters for 13 spermatological and 14 larval morphology traits are in most cases based on published records for congenics; however, if a family was fixed for one character, a reasonable assumption of monophyly was made in order that these data could be included.

Unfortunately, missing data remains for some taxa, e.g. *Pseudomunida fragilis* or *Kiwa hirsuta*. Information on developmental, larval and sperm morphology characteristics for these taxa will have to be added in the future.

2.5. Phylogenetic analysis

Maximum parsimony analyses were conducted using the program TNT (Tree analysis using New Technology, version 1.1, Goloboff et al., 2003) using both traditional and new technology searches for the partitioned dataset. Traditional heuristic searches were conducted using TBR branch swapping with 1000 random addition replicates and holding 10 trees at each step. New technology searches were replicated 10 times using different random seeds to examine that the shortest tree length was consistent, using Random and Consensus Sectarian Searches (RSS and CSS, respectively) and allowing for tree fusion. Default settings and optimality criteria were applied as recommended by Goloboff et al. (2008).

Analyses using Bayesian inference (BI) were conducted using MrBayes Version 3.1. (Huelsenbeck and Ronquist, 2001). The best-fit models of nucleotide evolution for each individual molecular partition (16S, 18S and 28S) were determined in Modeltest 3.7 (Posada and Crandall 1998) and using the Akaike Information Criterion (AIC). Then likelihood settings for each partition followed the GTR model with gamma distribution and invariant sites. Metropolis coupled Monte Carlo Markov Chains were run for between 5 and 15 million generations in two to three separate runs

until the split chain frequencies converged. Four differentially heated chains were run in each of two simultaneous runs, analyses were replicated twice. Topologies were sampled every 100 generations and free parameters were estimated during the run. The standard deviation of split frequencies converged to a value of between 0.003 and 4 for the separate runs for the combined analysis (0.002 for morphology partition alone and 0.005 for the molecular partition alone). After inspection of the likelihoods of the sampled trees, 25% of the generations were discarded as 'burn in'.

3. Results

3.1. Sequence data

The aligned combined dataset contained 64 taxa and 2939 characters of which 1098 are parsimony informative. Forty-two DNA sequences from 16 species were new (12 for 16S, 14 for 18S, and 12 for 28S). The morphological data comprised 121 parsimony informative characters.

The aligned 16S rRNA data comprised 467 positions of which 311 were variable (66.6%) with 241 parsimony informative (51.6%). The aligned 18S rRNA dataset comprised 2025 positions with 1140 variable sites (56%) of which 610 were parsimony informative (30%). The aligned 28S rRNA dataset contained 307 positions; 176 (57%) were variable of which 134 were parsimony informative (44%).

The 16S fragment was 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 (Chi = 216.02, df = 207, $P = 0.32$), 18S (Chi = 111.83, df = 207, $P = 1.00$), 28S (Chi = 87.33, df = 207, $P = 1.00$).

3.2. Analyses of the data partitions

3.2.1. Combined analysis of morphological and molecular partitions

The MP analysis resulted in eleven most parsimonious trees (CI = 0.446, RI = 0.661). The strict consensus for MP and the majority-rule consensus for BI analyses resolved two monophyletic clades: a combined Galatheidae + Porcellanidae clade and a clade containing the remaining taxa (Fig. 1). In the latter group, the Hippoidea, containing *Emerita*, *Lepidopa* and *Blepharipoda*, representing Hippidae, Lepidopidae and Blepharipodidae, respectively, is monophyletic and sister to the remaining Paguroidea, Lithodoidea, Chirostyliidae, Lomisidae, Aeglidae and Kiwidae. The Paguroidea + Lithodoidea are monophyletic (70% jackknife support, 1.0 posterior probability, Fig. 1) with a subdivision into a basal Pylochelidae and Parapaguridae, Paguridae + (Lithodidae + Hapalogastridae) and Diogenidae + Coenobitidae clades. The remaining clade supports *Lomis + Aegla* as sister taxon at the base to a clade including the Chirostyliidae with *Kiwa* placed internally.

Family-level support is strong throughout the tree with four notable exceptions: (1) Paguridae are rendered paraphyletic by an internally nested Lithodidae + Hapalogastridae, (2) *Paguristes* is not placed within the remaining diogenids, (3) the aforementioned placement of *Kiwa hirsuta* is nested within the Chirostyliidae between *Pseudomunida fragilis* and all other chirostyliids, (4) the Galatheidae are comprised of potentially three independent lineages with the *Leigalatheia* + Shinkainae + Munidopsinae and a *Galatheia* + *Allogalatheia* clades separated from all other galatheids and with the Porcellanidae nested between the latter two.

3.2.2. Separate analysis of morphological and molecular partitions

Analyses of the molecular partition under MP and BI were congruent with the combined analysis in supporting two main clades:

(1) Galatheidae + Porcellanidae, and (2) the Hippoidea at the base of a combined Paguroidea + Lomisoidea + Aegloidea + (Chirostyliidae + Kiwidae). The molecular partition also resolves the diogenids, pagurids, chirostyliids and galatheids as paraphyletic (Fig. 2). The key difference between the combined and the molecular trees is the significant paraphyly of the Paguroidea with ambiguous positions of the Parapaguridae and the Pylochelidae. The separate analyses using MP and BI placed these two paguroid families in a range of positions but never in a monophyletic clade with the remaining paguroids. Furthermore, the Pylochelidae were not recovered as monophyletic, with *Trizocheles* more closely related to the parapagurids, *Lomis* and *Aegla*, and *Pylocheles* basal to the paguroid-chirostyliid clade in both MP and BI analyses, albeit with variable support. The Parapaguridae were nested between the *Aegla + Lomis* clade and the chirostyliid-Kiwa clade in all cases, a relationship that was not supported in the combined analysis. The Chirostyliidae, with an internally nested *Kiwa*, retain their close association with *Aegla*, *Lomis* and the paguroids and are clearly removed from the proximity of Galatheidae and Porcellanidae. Therefore, results consistently support the combined analysis in rejecting a monophyletic Galatheoidea.

Separating nuclear genes (18S and 28S) from the mitochondrial 16S gene still retains the paraphyly; the nuclear genes further split the Paguroidea into separate Paguridae + Lithodidae and Diogenidae + Coenobitidae clades. They also indicate a closer link between the Chirostyliidae and the Porcellanidae, albeit with little support and while the Galatheidae remain far removed. A monophyletic Galatheoidea is not maintained in any case.

Analyses of the morphological partition were less consistent between approaches with the retention of polytomies in all cases (Fig. 2 shows results of the BI analysis). Chirostyliidae remain more closely related to the Paguroidea under BI (with 0.70 posterior probability). MP favoured the classic hypothesis of the Chirostyliidae sister to Porcellanidae + Galatheidae, though, with little nodal support. Nodal support was high for the derived taxa such as the hippoid and the lithodid clade and the Porcellanidae, and paguroid families are generally retained (although the Pylochelidae remain paraphyletic). *Aegla*, *Lomis* and *Kiwa* are placed together in the paguroid-lithodid-chirostyliid polytomy. The Chirostyliidae remain paraphyletic with the exclusion of *Eumunida* and *Pseudomunida* and Galatheidae is placed in a large polytomy that includes the porcellanids. Support for these respective clades is not strongly altered when only adult somatic characters are included (i.e. exclusion of sperm and larval characters).

In order to examine the morphological synapomorphies for the groups discussed, unambiguous character changes have been mapped on the combined analysis in Fig. 3 (Appendix 4).

4. Discussion

Although decapod phylogeny has attracted much recent interest (see Porter et al., 2005; McLaughlin et al., 2007; Ah Yong et al., 2009; Bracken et al., 2009; Chu et al., 2009 for recent reviews), only Ah Yong and O'Meally (2004) use a combined character approach, namely using adult somatic morphology and three genes. All remaining studies have applied either molecular, morphological, sperm or neurological characters, often with conflicting topologies. This has also been the case for studies restricted to Anomura and this study is therefore the first to provide a synthesis of phylogenetic data to evaluate anomuran phylogeny by combining molecular data with characters drawn from adult somatic morphology, sperm and larval morphology. The results show some agreement between combined and separate molecular and morphological analyses, namely the high support for the monophyletic Hippoidea, that *Aegla* and *Lomis* are recovered as sister taxa and a

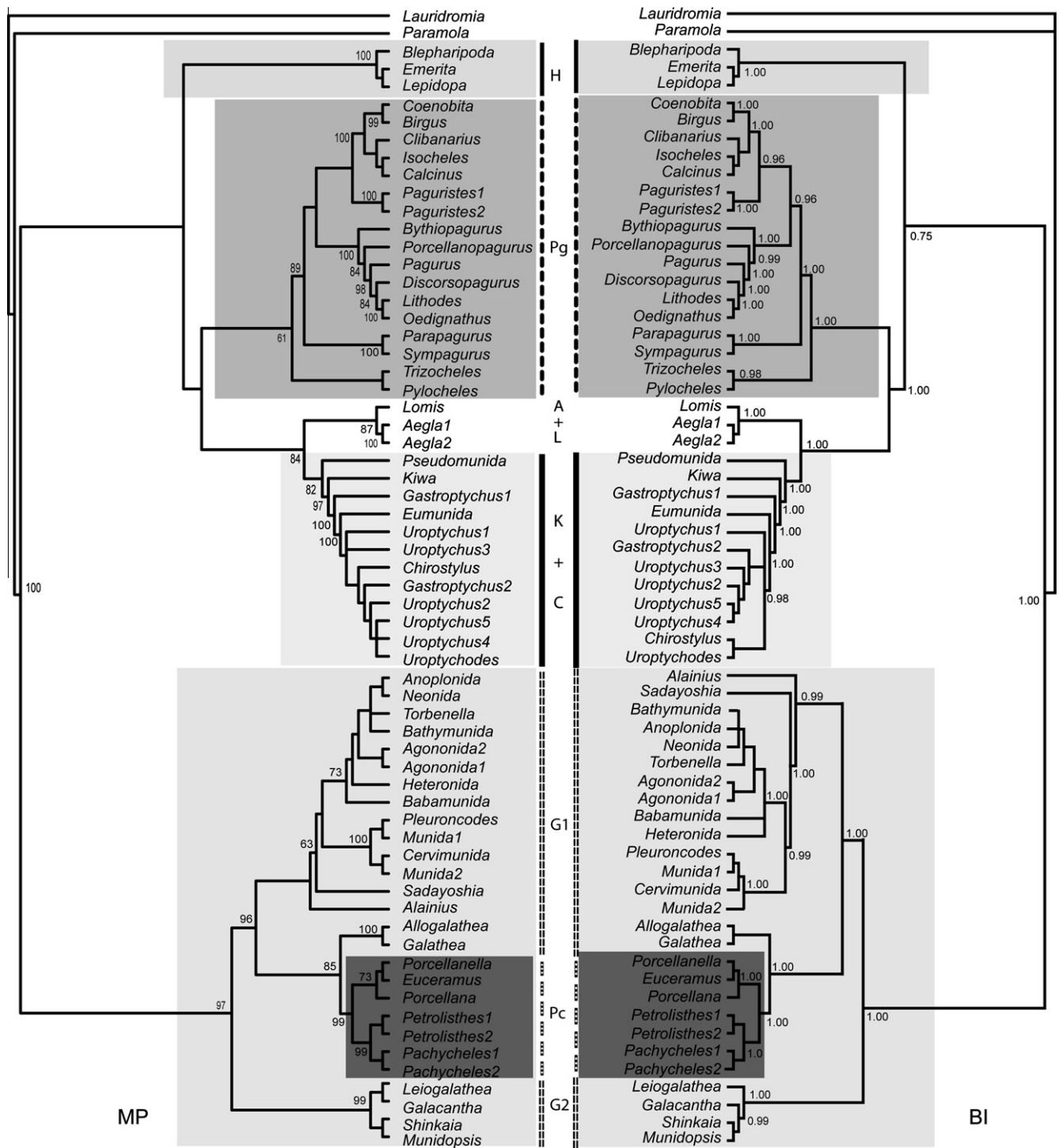


Fig. 1. Combined analyses of DNA (16S, 18S and 28S rRNA) and morphology: strict consensus of 11 MP topologies (left) and Bayesian topology (right). Abbreviations: H, Hippoidea, Pg, Paguroidea, L, Lomisidae, A, Aeglidae, K, Kiwaidae, C, Chirostylidae, G, Galatheidae, Pc, Porcellanidae. Jackknife support (MP) and Bayesian posterior probability values (BI) are indicated on branches.

consistent polyphyly of Galatheoidea. The Paguroidea remain inconsistently resolved with monophyly challenged in all but the combined analysis.

Galatheoidea *sensu lato*, including the squat lobsters (Galatheiidae and Chirostylidae) and the porcelain crabs (Porcellanidae), are unequivocally polyphyletic in the molecular and the combined molecular-morphology analyses with the Chirostylidae more closely related to the kiwaidae, aeglids, lomisisds and paguroids than to the remaining galatheoids.

Galatheoidea were previously unified based on a number of putative morphological synapomorphies. Scholtz and Richter (1995) separated the Anomura into two groups based on the presence or absence of the telson-uropod stretch receptor (TUSR), present in the 'Galatheoidea' + Hippoidea but absent from all other anomurans (citing Paul, 1989). Paul (1989) examined only two members of the family Galatheiidae (*Galathea strigosa* and *Munida quadrispina*) and 'Porcellanidae' but no members of the Chirostylidae. Paul (2003) subsequently concluded that, while the spiking

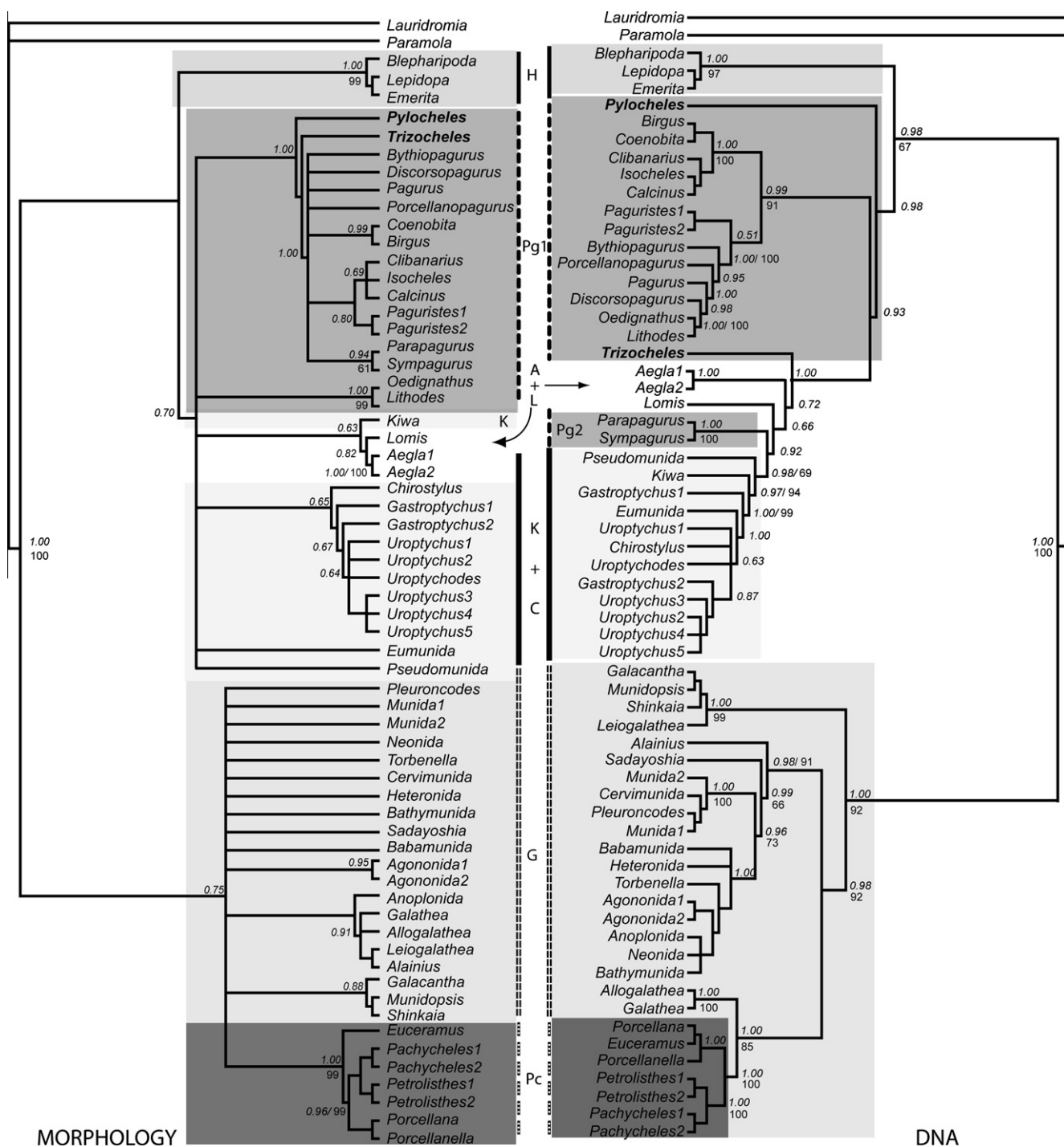


Fig. 2. Bayesian topologies for morphological (left) and molecular partitions (right). Values above nodes and italic are Bayesian posterior probability values, below nodes represent MP jackknife support. Abbreviations as in Fig. 1.

telson stretch receptor of the albuneids appears to be homologous with the galatheid TUSR, the characters and physiology of the hippid (*Emerita* spp. and *Hippa pacifica*) TUSR were concluded not to be homologous. No chirostyliids were included in that study, hence the telson stretch receptor cannot be considered a putative synapomorphy of the Galatheaidea *sensu lato* (i.e., inclusive of the chirostyliids) without further study. However, current results would predict the condition of the TUSR in Chirostyliidae to differ from that of Galatheaidae and Porcellanidae (character [36] was scored as unknown for Chirostyliidae in the morphology matrix).

Martin and Abele (1986) united Galatheaidea (Chirostyliidae + Galatheaidae + Porcellanidae) based on the shared presence of a phyllobranchiate gill structure (reported as dendrobranchiate but revised by McLaughlin et al., 2007) and gonopods (modified male pleopods). However, both characters are plesiomorphic. The former was used based on inclusion of *Aegla* in the Galatheaidea *s.l.* and phyllobranchiate gills are shared with the majority of Anomura and Brachyura, with exception of *Aegla* and blepharipodids, which have trichobranchiate gills (Dixon et al., 2003; McLaughlin et al., 2007).

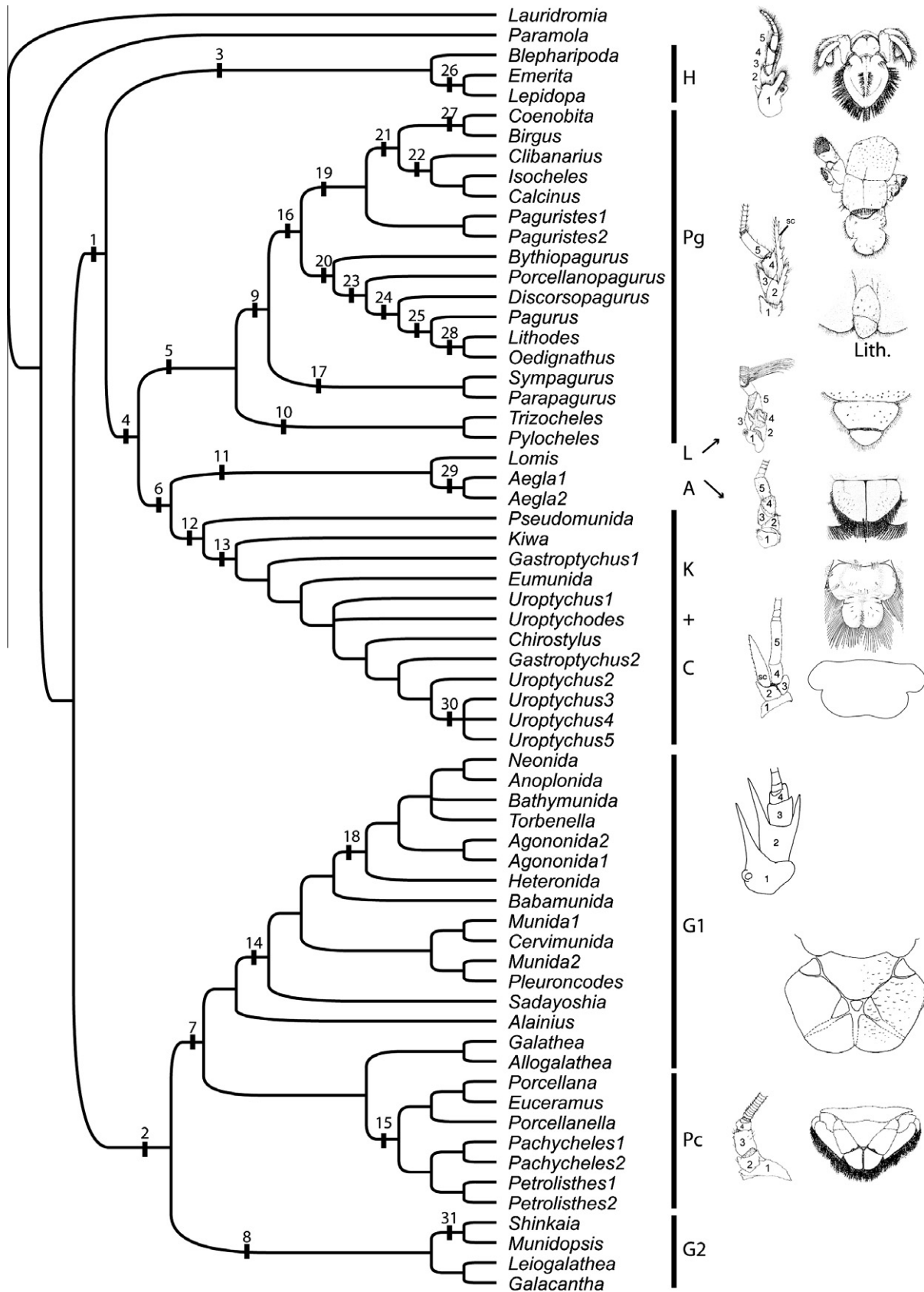


Fig. 3. One of 11 MP trees derived from combined morphological and molecular partitions showing nodes containing unambiguous character changes (see Appendix 4 for list of character state transformations). Antennal and telson morphologies after Martin and Abele (1986) except for *Aegla* sp. (Martin and Abele, 1988), *Kiwa hirsuta* (Macpherson et al., 2005), Galatheidae (Baba 2005) and chirostyliid telson (Schnabel, 2009). Abbreviations as in Fig. 1. Illustrations not to scale.

The modified male pleopods are also present in most decapods including Brachyura, most Astacidea, and Polychelida, some paguroids and *Lomis*. This character assignment is further complicated if the first and second gonopod are separately scrutinized; the first male pleopod is absent in porcellanids and in some galatheids, but present in most chirostylids; the second gonopod is present in all galatheids and chirostylids but only in some porcellanids. Hence, in addition to not being apomorphic, this character is not consistent within each family.

McLaughlin et al. (2007) reported only a single unifying apomorphy for Galatheoidea *s.l.*, the progressive development of the orbits. Ocular orbits are weakly to well-developed in brachyurans and mostly absent from anomurans. Ah Yong et al. (2009) argue that the orbital structure is linked to the presence of a well-developed rostrum, which is plesiomorphic and secondarily reduced in most anomuran families except for galatheids and chirostylids. McLaughlin et al. (2007) scored the Galatheidae as possessing a defined orbit as a result of the majority-rule scoring, but remarked that porcellanids and species of *Munida* and *Munidopsis* (the largest galatheid genera) vary between having no orbits and weak orbital development. All anomuran species examined here have at most weakly-developed orbits [character 44, state 1, see Appendix 2].

Evidence supporting a closer relationship of the Chirostylidae with the Paguroidea, based on sperm and larval morphology, has been noted in the past, but the taxonomic position of chirostylids as sister to the galatheids or porcellanids has never been revised (Clark and Ng, 2008; Guerao et al., 2006; Pike and Wear, 1969; Tudge, 1995, 1997). Clark and Ng (2008) strongly questioned whether chirostylids could be allied with galatheids and porcellanids based on larval morphology, but noted that the differences observed may be related to the generally abbreviated larval development in most chirostylids, making stage comparisons difficult. Guerao et al. (2006), however, resolved that larvae of *Eumunida annulosa* and *E. capillata* did not show abbreviated development as the other chirostylid genera, but that morphological characters were typically paguroid or even hippid, but not galatheid. For example, Paguroidea and Chirostylidae are united by the synapomorphic slender lobe on the scaphognathite that is lacking in other Galatheoidea (Van Dover et al., 1982) [character 121, node 4 in Fig. 3]. Additionally, Chirostylidae do not share the presence of larval posterolateral spines along the carapace that is a synapomorphy of all other Galatheoidea with exception of the genus *Munidopsis* (e.g., Clark and Ng, 2008; Guerao et al., 2006) [113]. Finally, a character not often illustrated but that may hold phylogenetic significance is the presence or absence of various numbers of terminal plumose setae on the antennal endopod with Galatheoidea having 0–1, Chirostylidae 2 and the majority of Paguroidea 3 [117].

Spermatozoal morphology also confirms a closer association between paguroids and chirostylids based on the shape of the acrosome vesicle (spherical or ovoid in chirostylids and paguroids and more elongate and fusiform in galatheids [99]) and possibly the possession of an acrosome ray zone [107] (Tudge, 1995, nodes 4 and 6 in Fig. 3).

Characters of adult morphology shared by chirostylids and paguroids are primarily plesiomorphies, e.g. the structure of the undivided telson (divided into plates in galatheids and porcellanids), the presence of the five-segmented antenna with or without scaphocerite (antennal scale), four-segmented and without scaphocerite in galatheids and porcellanids (Fig. 3 illustrates exemplars for each group). It is evident that Galatheidae and Porcellanidae, based on these synapomorphies alone, represent an independent trajectory and that the diverse adaptations in the remaining Anomura with subsequent divergent morphological character evolution have hitherto masked their close relationships.

Kiwa hirsuta forms a well-supported clade with the chirostylids according to the molecular data. This species was described from a single male specimen collected from deep-sea hydrothermal vents on the Galapagos Rift. Macpherson et al. (2005) deemed it sufficiently distinct to place it in its own family. Its close association with the chirostylids was noted by Macpherson et al. (2005) but the carapace shape, insertion of the fifth pereopod, anterior sternal characteristics and reduced eyes prompted them to exclude it from the Chirostylidae. In addition, Macpherson et al. (2005) supported their conclusion using a phylogenetic analysis of 18S rRNA sequences and six species representing the three families Chirostylidae, Galatheidae and Porcellanidae. Subsequently, McLaughlin et al. (2007) elevated *Kiwa* to the superfamily Kiwaoidea with results of a morphological study resolving it remote from the Chirostylidae. The present study sampled a significantly larger number of galatheoid (*s.l.*) taxa for a more rigorous examination of the position of *Kiwa* and its close affinity with the chirostylids cannot be denied. It shares the characteristic chirostylid synapomorphies of a transversely divided telson and absence of the eighth thoracic sternite. However, the morphological analysis across all characters remained inconclusive with *Kiwa* loosely aligned with either the *Lomis-Aegla* clade (BI, Fig. 2) or with the Chirostylidae (MP). While comparative analysis of the DNA sequence data unites *Kiwa* with the remaining chirostylids, *Pseudomunida fragilis* consistently remains at a basal position. Hence, the retention of *Kiwa hirsuta* in its own family renders the Chirostylidae paraphyletic unless *Pseudomunida* is elevated to family status itself. Until further examinations can corroborate the position of *Pseudomunida fragilis*, this species is retained in Chirostylidae.

A morphological character not previously considered may prove phylogenetically significant: a toothed mandibular cutting edge appears to unite the *Aegla* + *Lomis* + *Kiwa* + Chirostylidae clade [character 50, node 6 in Fig. 3]. In addition, the teeth are chitinous in *Kiwa*, *Aegla* and *Lomis*. The only other anomuran with this feature is the monotypic Pylojacquesidae (McLaughlin and Lemaitre, 2001). Pylojacquesidae was not included in the present study, but closer comparison of *Pylojaquesia colemani* with *Aegla*, *Kiwa* and *Lomis* also reveals a shared separation of the bases of the third maxilliped by a median projection, which McLaughlin and Lemaitre (2001) considered to be a convergent feature. They also concluded that *P. colemani* represents a potential link between the coenobitoid and paguroid clades and, based on results here, the link may extend further to the ‘chirostyloid–aegloid–lomisoid’ clade but remains to be resolved in future studies.

Combined and separate analyses strongly support a clade containing the Galatheidae and Porcellanidae. This clear relationship can be seen in a number of shared characteristic nucleotide substitutions and indels in the DNA sequence data. However, this close relationship becomes more ambiguous when morphological characters are included. Porcellanids comprise a distinct group that has undergone progressive ‘carcinisation’ (Borradaile, 1916), which includes an overall change of carapace shape from elongate to ovate or subovate and reduction of the tail with parallel loss of abdominal appendages. This alteration to the general body plan in conjunction with a number of porcellanid synapomorphies obscures the relationships to other anomurans, but nevertheless, a close morphological relationship between galatheids and porcellanids is undeniable. Both groups possess a telson subdivided into multiple calcified plates, a notably inflated basal article of the first antenna and second antenna with four instead of five articles (Fig. 3). Furthermore, comparative studies of sperm morphology indicate two synapomorphies that unite galatheids and porcellanids: the division of the inner acrosomal vesicle in the spermatozoa and the presence of unique septa in the perforatorial chamber, present in most examined galatheids and all porcellanids

but absent in all other anomurans (Jamieson and Tudge, 2000; Tudge, 1997; Tudge and Jamieson, 1996) (node 2, Fig. 3).

Overall, morphological characters alone clearly remain insufficient to fully resolve internal anomuran relationships, even with the inclusion of sperm and larval characters in addition to adult somatic morphology. However, this approach provides informative characters that can be used for defining clades supported in a combined molecular-morphological analysis.

5. New classification of squat lobsters

The paraphyly of the Galatheidae with the incursion of the Porcellanidae warrants further detailed investigations. The Galatheidae is represented by as many as three clades that may require elevation to families; more rigorous morphological examination is needed to define synapomorphies for diagnoses of each taxon.

As a consequence of topological position and the absence of unifying synapomorphies with other galatheids and porcellanids, Chirostyliidae are here excluded from the Galatheoidea and now included in the new superfamily Chirostyloidea.

5.1. Chirostyloidea Ortmann, 1892

Diagnosis: Body symmetrical, carapace with or without transverse striae, rostrum and supraocular spines present or absent. Sternal plastron consisting of sternites 3–7, thoracic somite 8 without sternal plate. Abdomen well-developed, all somites sclerotized, articulating. Tailfan well-developed, telson transversely divided by suture. Antennal peduncle consisting of five articles, antennal acicle present or absent. Mandible with serrated cutting edge. Maxilliped 3 lacking epipod. Pereopod 1 always chelate. Pereopod 2–4 as walking legs. Zoea 1 larva lacking posterolateral spines along the carapace; antennal endopod with two terminal plumose setae (characters not yet known in Kiwaidae).

Chirostyloidea contains the following families: Chirostyliidae Ortmann, 1892 and Kiwaidae Macpherson, Jones and Segonzac, 2005.

5.2. Galatheoidea Samouelle, 1819

Diagnosis: Body symmetrical. Rostrum well or weakly-developed, subtriangular, spiniform or broadly rounded; supraocular spines present or absent. Thoracic somite 8 distinct with sternal plate. Abdomen well-developed, all somites sclerotized, articulating. Tailfan well-developed, telson distinctly or indistinctly subdivided into several plates. Basal article of the first antenna notably enlarged. Antennal peduncle consisting of four articles (second and third of five articles fused). Mandible with entire cutting edge. Maxilliped 3 with or without epipod. Pereopod 1 always chelate. Pereopod 2–4 as walking legs. Sperm perforatorial chamber wall of the spermatozoa with prominent dividing septa. Inner acrosome zone of spermatozoa divided. Zoea 1 larva with or without (in Munidopsinae) posterolateral spines along the carapace; antennal endopod with 0–1 terminal plumose setae.

Galatheoidea contains the following families: Galatheidae Samouelle, 1819 and Porcellanidae Haworth, 1825.

6. Conclusion

Over recent years, evidence from multiple independent lines of research has cast increasing doubt on whether the chirostyliid squat lobsters are allied to other squat lobsters in the Galatheoidea. The synthesis of morphological (adult, larval and spermatological) and molecular data (ribosomal DNA sequences) with wide taxon sampling in the present study clearly shows the polyphyly of the

Galatheoidea *sensu lato*, resulting in recognition of a new superfamily, Chirostyloidea. However, other aspects of anomuran phylogeny remain ambiguous. These ambiguities primarily remain within the Paguroidea, but possible paraphyly within both the Galatheidae and Chirostyliidae will also require further attention in the future. Concurrent efforts to explore a wide array of other DNA markers for Decapoda (Chu et al., 2009; Tsang et al., 2008) will provide a stimulus for closer scrutiny of phylogenetic relationships that will need to be augmented by the search for (meaningful) morphological characters.

Note added in proof

Since this paper went to press, two studies revising the classification of the Chirostyloidea and Galatheoidea have appeared that expand on the new classification proposed herein (Ahyong et al., 2010; Schnabel and Ahyong, 2010). The family, Eumunidiidae A. Milne-Edwards and Bouvier, 1900, is added to the Chirostyloidea, and contains two genera (*Eumunida* and *Pseudomunida*) previously placed in the Chirostyliidae. Galatheoidea is now divided into four families, Galatheidae, Munididae, Munidopsidae and Porcellanidae, which correspond to the galatheoid clades recovered in Fig. 1 that contain *Galathea*, *Munida*, *Munidopsis* and *Porcellana*, respectively.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2010.11.011](https://doi.org/10.1016/j.ympev.2010.11.011).

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