The origin of king crabs: hermit crab ancestry under the magnifying glass

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The origin of king crabs from a hermit crab ancestor has caused controversy for more than a century. While the phylogenetic position of Lithodidae within the hermit crab family Paguridae has been strengthened in recent years, several key questions regarding the evolution of lithodid crabs have remained unanswered. In particular, it has been debated which hermit crabs constitute the closest extant relatives to lithodid crabs within Paguridae. Also, the relationships of the two lithodid subfamilies, Lithodinae and Hapalogastrinae, are unresolved. Answers to these questions are crucial to the understanding of the origin of king crabs, in particular which factors were the driving forces behind leaving a protective housing, transforming to a crab-like morphology and finally developing a large body size. To address these questions, we constructed the most comprehensive molecular phylogeny of Paguridae and Lithodidae to date. Our analyses revealed a species-rich clade of hermit crabs as closest relatives to lithodid crabs within Paguridae. Hermit crabs included in this clade have a predominantly shallow-water distribution in the North Pacific, agreeing with a proposed origin of lithodid crabs in this region. We suggest that the advances resulting from abandoning a shell-inhabiting lifestyle, rather than constraints of such shelters, played a central role in carcinization in this taxon. Phylogenetic relationships within Lithodidae revealed its two subfamilies to be non-monophyletic. Small-sized, shallow-water taxa are basal in the phylogenetic tree, while an increase in size and subsequent deep-sea distribution occurred later in the evolution of the group.

ADDITIONAL KEYWORDS: Crustacea – evolution – Lithodidae – molecular – Paguridae – phylogeny.

INTRODUCTION

King crabs (Lithodidae Samouelle, 1819) are anomuran crustaceans with a crab-like body, superficially resembling that of brachyuran (true) crabs. The evolutionary origin of the taxon has been discussed since the end of the 19th century when it was hypothesized that king crabs are secondarily calcified hermit crabs that left the protective gastropod housing and transformed to a crab-like form (Boas, 1880a, b; Bouvier, 1895). The process causing morphological modifications towards a crab-like body shape, with a reduced pleon, folded under a broadened and calcified cephalothorax, is termed carcinization (Borradaile, 1916; Scholtz, 2014). The hypothesis that lithodid crabs are derived from a hermit crab ancestor regained significant attention when investigated for the first time using molecular phylogenetic methods (Cunningham, Blackstone & Buss, 1992). Not only were king crabs placed within the Paguridae Latreille, 1802, but the molecules even suggested a nested position of the taxon within the hermit crab genus *Pagurus* Fabricius, 1775. All subsequent studies based on molecular data (Zaklan, 2002; Morrison *et al.*, 2002; Tsang *et al.*, 2008, 2011; Ahyong, Schnabel & Maas, 2009; Bracken *et al.*, 2009; Chu *et al.*, 2009; Schnabel, Ahyong & Maas, 2011; Bracken-Grissom *et al.*, 2013) as well as various morphological studies (Richter & Scholtz, 1994; Keiler & Richter, 2011; Reimann, Richter & Scholtz, 2011; Keiler, Richter & Wirkner, 2015) further supported the placement of lithodid crabs within the asymmetrical hermit crab family Paguridae.

Despite the growing evidence of a hermit crab ancestry of king crabs, McLaughlin *et al.* strongly opposed this

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evolutionary scenario (McLaughlin & Lemaitre, 1997; McLaughlin, Lemaitre & Tudge, 2004; McLaughlin, Lemaitre & Sorhannus, 2007; Lemaitre & McLaughlin, 2009). Reversal of complex characters related to dextral shell housings, like the morphology of the fourth pereopods, and a maladaptive scenario of a crab exposing the soft pleon made, in their view, this evolutionary pathway infeasible. Rather, the authors proposed the opposite evolutionary scenario, leading from king crabs to hermit crabs. The placement of lithodids within the Anomura has thus been heartily debated until recent years when steadily increasing evidence settled the position of the group within the Paguridae (Tsang et al., 2008, 2011; Ahyong et al., 2009; Chu et al., 2009; Schnabel et al., 2011; Bracken-Grissom et al., 2013).

The overall body shape of lithodid crabs is highly altered from its hermit crab ancestor. However, pagurid hermit crab asymmetries, influenced by inhabiting dextral gastropod shells (Palmer, 2004), such as pleon and claw asymmetries (McLaughlin & Lemaitre, 1997, 2000; McLaughlin et al., 2004; Duguid, 2010), are still present in lithodids (Tsang et al., 2011). Further traces of their pagurid origin can, for example, be found in the mouthparts (Boas, 1924; Jaszkowiak et al., 2015), setation (Keiler & Richter, 2011), internal organ organization (Anker & Paulay, 2013; Keiler et al., 2015) or vascular system (Keiler, Richter & Wirkner, 2013; Keiler et al., 2015).

Poor knowledge of phylogenetic relationships within the Paguridae (Matzen da Silva et al., 2011a) left the exact placement of lithodids within this hermit crab family uncertain. Various candidates have been highlighted as the possible closest extant relatives to lithodids within the Paguridae, yet conclusions from genetic studies so far have been restricted by limited taxon sampling. In early studies, king crabs were thought to be derived from an ancestor closely related to the genera *Nematopagurus* A. Milne-Edwards & Bouvier, 1892 and *Pylopagurus* A. Milne-Edwards & Bouvier, 1893 based on the presence of paired pleopods, found in females of these species and those of lithodids (Boas, 1924). Reimann et al. (2011), based on a cladistic analysis, also found a sister relationship of these genera to lithodids within the remaining Paguridae. An exclusively North Pacific genus, *Discorsopagurus* McLaughlin, 1974, has drawn attention as the possibly closest relative in recent studies (Morrison et al., 2002; Ahyong et al., 2009; Schnabel et al., 2011; Bracken-Grissom et al., 2013). *Discorsopagurus* inhabits non-coiled housings and has an almost symmetrical pleon (Komai, 2003), as also found in male lithodids. Other studies found a sister relationship with one or few other exclusively North Pacific genera (*Labidochirus* Benedict, 1892; *Elassochirus* Benedict, 1892; and *Pagurodofleinia* Asakura, 2005), as well as part of the genus *Pagurus* (Cunningham et al., 1992; Tsang et al., 2008, 2011; Chu et al., 2009). In particular, a possibly nested position of Lithodidae within *Pagurus* has been highlighted (Cunningham et al., 1992). *Pagurus* is a species-rich genus, and various informal morpho-groups have been established (Forest & de Saint Laurent, 1968; McLaughlin, 1974; Ingle, 1985; Lemaitre & Cruz-Castaño, 2004). Some of these informal groupings have been confirmed using molecular markers (Matzen da Silva et al., 2011a; Olguín & Mantelatto, 2013), and a highly polyphyletic pattern of the genus has been indicated (Cunningham et al., 1992; Reimann et al., 2011; Bracken-Grissom et al., 2013).

Lithodidae are only found in temperate regions and deep-sea habitats. Few genera have a global distribution via the deep sea, while the largest number of lithodid genera is restricted to the North Pacific, where they display a high morphological diversity (Stevens & Lovrich, 2014). From the distribution pattern, combined with physiological and phylogenetic data, it has been concluded that lithodid crabs originated in the shallow North Pacific (Makarov, 1938; Zaklan, 2002; Hall & Thatje, 2009b). While the shallow-water genera of the North Pacific are monotypic or only contain few species, the king crab genera *Lithodes* Latreille, 1806; *Neolithodes* A. Milne-Edwards & Bouvier, 1894; and *Paralomis* White, 1856 are species rich, and new species have been frequently discovered in the last decades (Williams, Smith & Baco, 2000; Macpherson, 2001, 2003, 2004; Takeda & Nagai, 2004; Ahyong & Dawson, 2006; Spiridonov et al., 2006; Takeda & Bussarawit, 2007; Macpherson & Chan, 2008; Hall & Thatje, 2009a; Guzmán, 2009; Ahyong, 2010a, b; Ahyong & Chan, 2010; Muñoz & García-Isarch, 2013). The deep-sea lineages diversified on a global scale and constitute the majority of today's species diversity of Lithodidae (Hall & Thatje, 2009b). Lithodidae exhibit a wide range of morphological diversity, ranging from small-sized species, such as *Hapalogaster* Brandt, 1850; *Dermaturus* Brandt, 1850; or *Cryptolithodes* Brandt, 1848, to the large box and king crabs. Balss (1924) pointed out the similarities between different body shapes of lithodids and brachyuran crabs as prime examples for convergent evolution. The gross morphology of the lithodid genus *Cryptolithodes*, for example, superficially resembles that of the brachyuran genus *Aethra* Latreille in Cuvier, 1816. The large king crabs on the other hand, in particular the genus *Lithodes*, resemble the brachyuran spider crabs of the genus *Maja* Lamarck, 1801.

Knowledge of internal relationships within the Lithodidae is limited, and the association of the two lithodid subfamilies, Hapalogastrinae Brandt, 1850 and Lithodinae Samouelle, 1819, is uncertain (Hall & Thatje, 2009b; Bracken-Grissom et al., 2013). Bracken-Grissom et al. (2013) recovered different relationships

between taxa of the two subfamilies, depending on their analyses. Using only molecular data, neither taxa were found to be monophyletic, while including morphological data in their analyses resolved both taxa as monophyletic. Hall and Thatje (2009a) resolved both subfamilies as monophyletic, but with a poorly supported placement of *Cryptolithodes* (Hapalogastrinae). Recently, *Cryptolithodes* was, however, placed outside Lithodinae and within Hapalogastrinae based on molecular data (Thatje & Hall, 2016). The two subfamilies are separated by the calcification of the third to fifth tergites of the pleon in Lithodinae or lack thereof in Hapalogastrinae (McLaughlin, 2014). *Cryptolithodes*, however, is the most heavily calcified lithodid crab, and its ambiguous phylogenetic placement makes the use of the degree of pleon calcification as an autapomorphy for the two subfamilies uncertain. Information on basal lithodid relationships is crucial for understanding the evolutionary pathway of lithodid crabs and the driving forces leading from a shell-inhabiting to a free-living lifestyle via the process of carcinization.

In the present study, we construct an extensive molecular phylogeny of Paguridae and Lithodidae using nuclear ribosomal and mitochondrial genes to cover a suitable range of genetic variability (Toon *et al.*, 2009). New and available sequence data are combined to construct the most comprehensive phylogeny of pagurid hermit crabs and lithodid crabs to date in the search for the closest living relatives of Lithodidae within the hermit crabs.

MATERIAL AND METHODS

TAXON SAMPLING

New sequences from 40 species of Paguridae and Lithodidae were obtained for this study. Specimens were obtained both in the field and from museum collections (Table 1). Collection of new material focussed on the temperate regions of the Northern Hemisphere, in particular the North Pacific. This region has been highlighted as the region of origin of Lithodidae (Hall & Thatje, 2009b), and the closest extant relatives to lithodids are likely found in this region. The data set was complemented by a large range of taxa of Paguridae and Lithodidae with sequences available in GenBank and the Barcode of Life Data Systems (BOLD), to cover a broad range of taxa and geographic regions (Table 1). Six representatives of the genera *Paguristes* and *Areopagurites* (family Diogenidae Ortmann, 1892) were chosen as outgroup taxa, based on previous studies that indicated these genera as closest relatives to Paguridae and Lithodidae (Morrison *et al.*, 2002; Bracken-Grissom *et al.*, 2013).

MOLECULAR WORK

Extraction

Specimens collected for this study were preserved in 96% ethanol prior to DNA extraction. Molecular work was conducted in the Biodiversity Laboratories, University of Bergen, Norway. Total genomic DNA was extracted from muscle tissue using a Gene Mole automatic nucleic acid extractor from Mole Genetics AS, Norway, or using the Qiagen DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA, USA), following the manufacturer's standard protocols.

PCR and sequencing

Sequences of five genes were amplified by PCR: three mitochondrial markers [ribosomal 12S and 16S rRNA subunits, and cytochrome *c* oxidase 1 (*COI*)] and two nuclear markers (18S and 28S rRNA subunits). PCR reactions were carried out on a Bio-Rad C1000 Thermal Cycler using Takara polymerase in 25-µL reactions. Primers from previous studies as well as newly designed primers were used (Table 2). The 28S gene was amplified using two primer pairs, resulting in two overlapping fragments. For some species, the 12S and *COI* genes, situated next to each other in the mitochondrial genome, were amplified in a single PCR run, using the primers 12S-A-Paguridae and COI-B-Paguridae. All PCR products were checked for successful amplification on 1% agarose gels stained with GelRed. PCR products were purified and sequenced in both directions at Macrogen Inc. using the same primers as for amplification. For the 18S fragment two additional primers (18S-A– and 18S-B+) were used for sequencing the entire PCR product. Contigs were assembled using Lasergene SeqMan Pro 8.1. To exclude a possible presence of pseudogenes or gene duplicates, the individual sequence chromatogram files were checked for the presence of double peaks, and the *COI* alignment was translated into amino acids and checked for premature stop codons and frame shifts using BioEdit 7.2.3. Sequences are deposited in GenBank (Table 1).

Sequence alignments

The data set included 49 Lithodidae and 69 Paguridae species, as well as six Diogenidae species as outgroup taxa. Sequences for *COI*, 16S, 12S and 18S were aligned in eBioX 1.5 using the MUSCLE algorithm (Edgar, 2004). 28S sequences were aligned in the online version of MAFFT 7 (Katoh & Standley, 2013) using the E-INS-i strategy. Individual alignments were checked by eye. The *COI* alignment was checked by translation into amino acids using Seaview 4.4 with the genetic code set to 'Invertebrate mt'. Sequences

Table 1. Taxa included in this study with list of GenBank and BOLD accession numbers of the molecular marker. Accession numbers of new sequences are indicated with an asterisk

Taxa	16S	18S	28S	*COI*	12S
Family Diogenidae					
Areopaguristes hewatti	KF182535	KF182482	KF182644	–	–
Areopaguristes hummi	KF182542	KF182484	KF182641	–	–
Paguristes cadenati	KF182540	KF182493	KF182637	–	–
Paguristes puncticeps	KF182538	KF182487	KF182639	–	–
Paguristes triangulatus	KF182539	KF182489	KF182638	–	–
Paguristes turgidus	AF436056	AF436020	AF435997	DQ882097	–
Family Lithodidae					
Subfamily Hapalogastrinae					
Acantholithodes hispidus	–	–	–	DQ882026	–
Hapalogaster dentata	AF425327	–	AF425347	AF425306	AF425306
Hapalogaster grebnitzkii	KY426325*	–	–	–	KY426292*
Hapalogaster mertensii	AF425328	KF182451	KF182601	KY426267*	AF425307
Oedignathus inermis	AF425334	–	AF425313	AF425353	AF425313
Placetron wosnessenskii	KY426329*	–	KY454171*	DSALA006-06	–
Subfamily Lithodinae					
Cryptolithodes sitchensis	AF425324	KF182453	KF182603	KC107820	AF425303
Cryptolithodes typicus	AF425325	AF436019	AF425345	AF425304	AF425304
Glyptolithodes cristatipes	AF425326	–	AF425346	AF425305	AF425305
Lithodes aequispinus	KY426332*	–	KY454183*	AF425308	AF425308
Lithodes confundens	HM020949	–	FJ462642	HM020901	–
Lithodes couesi	–	–	–	DQ882086	–
Lithodes ferox	HM020950	–	HM020856	KY426276*	KY426296*
Lithodes longispina	–	–	–	AB476815	–
Lithodes maja	KY426333*	KY454206*	AF425350	FJ581746	AF425309
Lithodes murrayi	HM020954	–	HM020857	HM020899	–
Lithodes nintokuae	AB769476	–	–	AB769476	AB769476
Lithodes paulayi	–	–	–	GU289677	–
Lithodes santolla	KF182572	AF439385	KF182602	KY426275*	AF425310
Lithodes turkayi	EU493268	–	–	KC196529	–
Lithodes turritus	KJ132573	–	–	–	–
Lopholithodes foraminatus	KY426330*	–	KY454182*	DQ882088	KY426295*
Lopholithodes mandtii	AF425333	KY454205*	AF425352/KY454174*	KY426271*	AF425312
Neolithodes asperrimus	HM020940	–	HM020847	HM020891	–
Neolithodes diomedeae	–	–	–	KC196528	–
Neolithodes duhameli	HM020946	–	HM020849	HM020892	–
Neolithodes grimladii	–	–	–	JQ305973	–
Neolithodes nr. *brodiei*	HM020942	–	FJ462640/HM020888	HM020894	–
Paralithodes brevipes	AF425337	–	AF425356	NC021458	AF425316
Paralithodes camtschaticus	AF425338	JN192147	AB193823	JF738154	AF425317
Paralithodes platypus	KY426328*	JN192152	AB193821	KY426274*	KY426297*
Paralomis aculeata	HM020958	–	HM020862	HM020904	–
Paralomis africana	EU493275	–	HM020864	HM020907	–
Paralomis anamerae	HM020959	–	HM020865	HM020906	–
Paralomis birsteini	KY426326*	–	HM020867	EU493260	KY426294*
Paralomis cristata	EU493267	–	–	HM020911	–
Paralomis cristulata	EU493271	–	HM020870	HM020908	–
Paralomis dofleini	HM020962	–	HM020871	HM020912	–
Paralomis elongata	–	–	HM020872/HM020887/HM20884	HM020914	–
Paralomis erinacea	HM020966	–	HM020873	HM020915	–

Table 1. *Continued*

Taxa	16S	18S	28S	*COI*	12S
Paralomis formosa	HM020971	–	FJ462641/HM020886	HM020918	–
Paralomis granulosa	AF425339	–	AF425358/HM020877	AF425318	AF425318
Paralomis hirtella	KY426327*	–	–	KY426272*	KY426293*
Paralomis multispina	–	–	–	AB211296	–
Paralomis pacifica	–	–	–	AB476750	–
Paralomis spinosissima	HM020982	–	HM020879	HM020927	–
Paralomis zealandica	HM020980	–	–	HM020935	–
Phyllolithodes papillosus	AF425340	KY454204*	AF425359/KY454175*	KY426273*	AF425319
Rhinolithodes wosnessenskii	KY426331*	–	AF425360	AF425320	AF425320
Family Paguridae					
Agaricochirus alexandri	–	KF182447	KF182593	–	–
Anapagurus breviaculeatus	KY426316*	–	KY454162*	KY426262*	KY426286*
Anapagurus chiroacanthus	KY426315*	KY454187*	KY454161*	KY426263*	KY426285*
Anapagurus hydmanni	–	KJ182993	–	KJ183012	–
Anapagurus laevis	KY426317*	KY454186*	KY454163*	BNSC284-11	KY426287*
Cestopagurus timidus	KY426314*	KY454192*	KY454159*	KY426261*	KY426288*
Discorsopagurus schmitti	AF436055	AF436017	KY454176*	KY426283*	KY426298*
Elassochirus cavimanus	KY426342*	–	–	KY426281*	KY426302*
Elassochirus gilli	KY426343*	–	–	KY426282*	KY426300*
Elassochirus tenuimanus	KY426341*	KY454198*	KY454184*	KY426279*	KY426301*
Labidochirus splendescens	AF425332	–	AF425351	–	AF425311
Manucomplanus ungulatus	KF182575	KF182457	KF182612	–	–
Nematopagurus gardineri	–	–	–	MDECA670-10	–
Nematopagurus longicornis	KY426318*	KY454188*	KY454169*	KY426264*	KY426289*
Nematopagurus meiringae	–	–	–	MDECA669-10	–
Paguridium minimum	KY426319*	KY454191*	KY454168*	–	KY426308*
Pagurus acadianus	–	–	–	FJ581812	–
Pagurus alatus	KY426323*	–	JN107619	KY426270*	KY426309*
Pagurus aleuticus	KY426340*	–	KY454180*	KY426280*	KY426299*
Pagurus arcuatus	–	–	–	FJ581817	–
Pagurus armatus	–	–	–	AF483159	–
Pagurus beringanus	KY426337*	KY454201*	KY454173*	KY426277*	KY426307*
Pagurus bernhardus	KY426339*	KY454197*	JN107623/KY454185*	JN107580	AF425314
Pagurus brachiomastus	–	–	–	JN5990075	–
Pagurus brevidactylus	KF182563	KF182495	KF182610	–	–
Pagurus bullisi	KF182568	KF182454	KF182595	–	–
Pagurus caurinus	KY426336*	KY454200*	KY454181*	KY426278*	KY426306*
Pagurus chevreuxi	KY426312*	-	KY454160*	–	–
Pagurus comptus	FJ869145	KY454202*	KY454170*	KY426265*	KY426290*
Pagurus criniticornis	DQ369947	–	–	–	–
Pagurus cuanensis	KY426322*	KY454190*	JN107625	JN107584	KY426310*
Pagurus edwardsii	FJ869146	–	–	CFAD141-11	–
Pagurus excavatus	JN107610	–	JN107628	JN107587	–
Pagurus exilis	FJ869147	–	–	-	–
Pagurus forbesii	KF962984	–	–	KF962980	–
Pagurus forceps	FJ869150	–	–	–	–
Pagurus gladius	JX238503	–	–	–	–
Pagurus granosimanus	KY426338*	KY454196*	KY454178*	GU442314	KY426305*
Pagurus hirsutiusculus	KY426334*	KY454193*	KY454177*	GU442400	AF425315
Pagurus kennerlyi	KY426345*	KY454195*	KY454172*	KY426284*	KY426304*
Pagurus leptonyx	DQ369946	–	–	–	–
Pagurus longicarpus	AF150756	AF436018	AF425343/AY739185	AF150756	AF150756

Table 1. *Continued*

Taxa	16S	18S	28S	*COI*	12S
Pagurus maclaughlinae	KF182566	KF182460	KF182611	–	–
Pagurus mbizi	KY426320*	–	KY454167*	–	KY426311*
Pagurus minutus	–	–	–	JX502978	–
Pagurus nr. *carolinensis*	KF182565	KF182465	KF182609	–	–
Pagurus ochotensis	KY426335*	KY454199*	KY454179*	JN590062	–
Pagurus pectinatus	–	–	–	JN5990060	–
Pagurus perlatus	JQ805783	–	–	–	–
Pagurus pollicaris	FJ869152	KF182458	KF182589	AF483163	–
Pagurus prideaux	KY426321*	KY454189*	JN107629	JQ306249	–
Pagurus provenzanoi	FJ869154	–	–	–	–
Pagurus proximus	–	–	–	KC347562	–
Pagurus pseudosculptimanus	KF962986	–	KY454165*	KY426268*	–
Pagurus pubescens	KY426344*	KY454194*	JN107633	JQ305956	KY426303*
Pagurus pubescentulus	KY426324*	–	KY454166*	KY426269*	–
Pagurus samuelis	–	–	–	GU443022	–
Pagurus similis	–	–	–	HM180751	–
Pagurus stimpsoni	KF182564	KF182466	KF182613	–	–
Pagurus venturensis	–	–	–	GU442190	–
Pagurus villosus	FJ869155	–	–	CFAD136-11	–
Phimochirus holthuisi	KF182578	KF182455	KF182594	–	–
Phimochirus randalli	KF182577	KF182450	KF182591	–	–
Pylopaguridium markhami	KF182570	KF182478	KF182597	–	–
Pylopagurus discoidalis	KF182569	KF182496		–	–
Spiropagurus elegans	KY426313*	KY454203*	KY454164*	KY426266*	KY426291*
Spiropagurus profundorum	–	–	–	MDECA610-10	–
Tomopagurus merimaculosus	KF182567	KF182497	KF182590	–	–

Table 2. Primers used for PCR amplification and sequencing

Marker	Primer	Primer sequence (5′–3′)	Reference
COI	HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer *et al.* (1994)
	LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer *et al.* (1994)
	COI-A-Paguridae	TCT TAT ATT TCC ACT ATA AAG CC	This study
	COI-B-Paguridae	ATT CTT GAC TTA CAA TRT GTG A	This study
16S	LR-N-13398	CGC CTG TTT AAC AAA AAC AT	Simon *et al.* (1994)
	LR-J-12887	CCG GTC TGA ACT CAG ATC ACG T	Simon *et al.* (1994)
	16S-A-Paguridae	AAG ATA GAA ACC AAC CTG GCT C	This study
	16S-B-Paguridae	TGC CTG TTT AAC AAA AAC ATG TC	This study
12S	12S-A-Paguridae	ATT ATA ATA GGG TAT CTA ATC CTA G	This study
	12S-B-Paguridae	AAT GTT CCA ATR TCT TTA TGG	This study
18S	18S-329	TAA TGA TCC TTC CGC AGG TT	Spears *et al.* (1992)
	18S-328	CCT GGT TGA TCC TGC CAG	Spears *et al.* (1992)
	18S-A– (sequencing)	CAG CMG CC GCG GTA ATW C	Spears *et al.* (1992)
	18S-B+ (sequencing)	ATT CCC CGT TAC CCG	Spears *et al.* (1992)
28S	28S-OI	GCG GAG GAA AAG AAA CTA AC	Zaklan (2001)
	28S-R443	CCT CAC GGT ACT TGT TCG CTA TCG G	Ahyong *et al.* (2009)
	28S-Paguridae-F1	CGT AGA GTC GGG TTG CTT GA	This study
	28S-Paguridae-R1	CTT TCG GGT CCC AAC ATG TC	This study

downloaded from GenBank were cut to the corresponding sequence region of our own PCRs. BLAST searches of sequences that appeared suspicious in the alignments revealed few published sequences to be contaminations, which therefore were removed from the data set. Those sequences are 28S for *Pylopagurus discoidalis* (A. Milne-Edwards, 1880) (KF182614); *COI* for *Nematopagurus squamichelis* Alcock, 1905 (KJ150706); and *COI* for *Pylopaguropsis magnimanus* (Henderson, 1896) (KM043479). A 16S sequence attributed to *Cestopagurus timidus* (Roux, 1830) (FR849637) available on GenBank is misidentified and appears to belong to *Pagurus prideaux* Leach, 1815 based on comparison with our data from these species. The 18S sequence of *Pylopaguridium markhami* McLaughlin & Lemaitre, 2001 (KF182478) was trimmed, since the end of the sequence consists of a repetition of a previous section of the same sequence, possibly generated during processing of the sequence data. The 18S sequence of *Oedignathus inermis* (Stimpson, 1860) (Z14062) was excluded due to few obvious minor sequencing errors in highly conservative regions. The 18S (KF182453) and 28S (KF182603) sequences of *Cryptolithodes* sp. were assigned to *Cryptolithodes sitchensis* Brandt, 1853, based on the 16S gene of *Cryptolithodes* sp. from the same study (KF182574) (Bracken-Grissom *et al.*, 2013), which is identical to sequences of *C. sitchensis* from other studies. GenBank sequences attributed to *Neolithodes brodiei* Dawson & Yaldwyn, 1970, sampled from Vanuatu (Snow, 2010) appear to come from an undescribed species (Ahyong, 2010b) and are here referred to as '*Neolithodes* nr. *brodiei*'. The alignments of the non-protein coding genes were subsequently run in Gblocks 0.91b (Castresana, 2000) to exclude ambiguous aligned regions, using the Gblocks server. Gblocks criteria used for this were for a less stringent selection, allowing for gaps within blocks, smaller final blocks and less strict flanking positions. The 28S alignment was not complete for all taxa over the entire length. Gblocks treats missing data like gaps and would, therefore, also remove highly conserved regions in this alignment. Ambiguously aligned regions in 28S were removed by hand, using the same parameters as Gblocks, but considering only gaps. The single gene alignments were concatenated to a single file using MacClade 4.06. PartitionFinder 1.1.1 (Lanfear *et al.*, 2012) was used to determine the best partitioning scheme and best-fit nucleotide substitution models for the concatenated data set under the Bayesian information criterion. The 'greedy' algorithm was used with branch lengths of alternative partitions 'linked'. The analysis suggested a partitioning of the data set by each marker as well as each codon position for the protein-coding *COI*. PartitionFinder suggested as the best-fit substitution models SYM + I + G for the first codon position of *COI* and 18S, F81 for the second

codon position of *COI*, GTR + G for the third codon position of *COI*, GTR + I + G for 16S and 28S, and HKY + I + G for 12S.

Phylogenetic analyses

The concatenated data set was analysed using maximum likelihood (ML) and Bayesian inference (BI) approaches. The ML analysis were conducted using RAxML 8.2.4 (Stamatakis, 2014), on the CIPRES science gateway (Miller, Pfeiffer & Schwartz, 2010). A unique GTR model of sequence evolution was specified for each partition following the scheme given by PartitionFinder with corrections for a discrete gamma distribution for site-rate heterogeneity (GTRGAMMA). The GTRCAT model was used for the bootstrapping phase. Thousand rapid bootstrap iterations were conducted to search for the best-scoring ML tree in one single program run. BI was conducted in MrBayes 3.2.2 (Ronquist *et al.*, 2012), on the Lifeportal, University of Oslo. The concatenated data set was partitioned following the scheme given by PartitionFinder. Each partition was run under the best-fit model of evolution, and all model parameter values were 'unlinked' among partitions. Two independent runs using four Metropolis-coupled Markov chain Monte Carlo analyses were performed. The chains were run for 20 million generations and sampled every 500 generations. The first 10 000 trees were discarded as burn-in, and a 50% majority-rule consensus tree was obtained from the remaining saved trees. The average standard deviation of split frequencies was checked for convergence towards zero, and MrBayes parameter files were examined in Tracer 1.6 (Rambaut *et al.*, 2014) to assess if runs had reached a stationary phase and converged on model parameters.

A second data set was produced, based on the phylogenetic tree obtained from the initial analyses, comprising only representatives of Lithodidae and pagurid hermit crabs that had been identified as sister clade to Lithodidae, as well as one outgroup taxon (*Pagurus comptus* White, 1847). Ambiguously aligned positions of the non-protein-coding gene alignments were removed as described for the initial data set. Since the gene alignments with this limited number of taxa contained fewer ambiguous positions, the resulting alignments were longer and contained more phylogenetic information. The single gene alignments were concatenated, and PartitionFinder was used as described above. The analyses suggested the same partition scheme as in the previous data set, with the same best-fit substitution models, except F81 + I for the second codon position of *COI*, HKY + I + G for 16S and K80 + I for 18S. The data set was analysed using ML and BI as described for the initial data set. The resulting phylogenetic trees were visualized using Dendroscope 3.2 (Huson & Scornavacca, 2012).

RESULTS

The phylogenetic trees obtained from ML and BI analyses were largely corresponding. Support values, however, were lower in the trees resulting from the ML analyses than in the trees obtained by BI (Figs 1, 2). We included the mitochondrial 12S and 16S rRNAs and the mitochondrial cytochrome *c* oxidase 1 (*COI*), as well as parts of the nuclear 18S and 28S rRNAs. All these genes have previously been proven to be useful in systematic studies of crustaceans (Schubart, Neigel & Felder, 2000). Different rates of evolution among the genes make a concatenated set of these markers a valuable phylogenetic tool for resolving a range of taxonomic levels (Toon *et al.*, 2009). Additional sequences from a large range of pagurid and lithodid species were downloaded from GenBank and BOLD, allowing us to compile the most complete data set to date, in the search for the closest extant relatives of lithodid crabs. The resulting data set was fragmentary for many taxa, since for most species only one or few genes were available in GenBank and BOLD. Deep phylogenetic nodes were resolved by taxa with a larger coverage in the matrix, while species with low coverage, for example only *COI*, clearly affiliated with taxa that had a larger coverage, usually congeneric species.

Our results show a monophyletic Lithodidae, deeply nested within a paraphyletic hermit crab family Paguridae (Fig. 1). Paguridae with the contained Lithodidae was found clearly distinct from the diogenid outgroup taxa (Fig. 1).

A clade of pagurid hermit crabs was clearly resolved as the sister taxon to Lithodidae within the Paguridae, which we refer to as 'pagurid–lithodid sister clade' (PLS clade) (Fig. 1). This clade shared the last common ancestor (LCA) with lithodid crabs within the hermit crabs. All species included in this clade are shallow-water hermit crabs, mainly distributed in the North Pacific. It consists of some genera exclusively found in the North Pacific: *Discorsopagurus*, *Elassochirus* and *Labidochirus*, as well as various species of *Pagurus*. Included representatives of *Pagurus* in the PLS clade belong to several of the established informal *Pagurus* morpho-groups; the 'bernhardus', 'trigonocheirus', 'capillatus' and part of the 'comptus' group (Forest & de Saint Laurent, 1968; McLaughlin, 1974; Lemaitre & Cruz-Castaño, 2004), as well as species that have not been assigned to any of these informal groupings. *Pagurus* species included in this PLS clade are also exclusive to the North Pacific, except for four species from the North Atlantic, which have close related species in the Pacific.

The usage of *Pagurus* as a catch-all genus for species with a general pagurid hermit crab morphology is well illustrated in the phylogenetic tree (Fig. 1), as representatives of the genus are dispersed throughout the family. The analyses largely confirm previously established informal *Pagurus* groups (Fig. 1). Only representatives of the 'comptus' group show a clear separation between species from South America ('comptus' group I) and the North Pacific ('comptus' group II). Two *Pagurus* clades, corresponding to the 'provenzanoi' group and subdivision I by Ingle (1985), also contain species that are assigned to other genera, *Manucomplanus* McLaughlin, 1981 and *Paguridium* Forest, 1961, respectively. Except for *Pagurus*, all other hermit crab genera included in the phylogenetic analyses with multiple representatives were resolved as monophyletic.

While Lithodidae was resolved as monophyletic overall, the two subfamilies Lithodinae and Hapalogastrinae appear not to be monophyletic (Fig. 2). Basal to the remaining lithodid taxa are the two hapalogastrine genera *Oedignathus* Benedict, 1895 and *Hapalogaster* (Fig. 2). The lithodine genus *Cryptolithodes* nests among the hapalogastrine and likely forms the sister taxon to all remaining lithodids, including all Lithodinae and the two monotypic hapalogastrine genera *Placetron* Schalfeew, 1892 and *Acantholithodes* Holmes, 1895.

The internal phylogeny of the Lithodidae is not fully resolved in our analyses, especially with respect to the genus *Paralithodes* Brandt, 1848. A sister relationship between the monotypic genera *Rhinolithodes* Brandt, 1848 and *Phyllolithodes* Brandt, 1848 is highly supported. The genus *Paralomis* forms a highly supported clade, also including the monotypic genus *Glyptolithodes* Faxon, 1895. Sister taxon to *Paralomis*/*Glyptolithodes* is the genus *Lopholithodes* Brandt, 1848, which consists of only two species that were both included in the analyses. Another highly supported clade consists of *Lithodes* and *Neolithodes*.

DISCUSSION

INTERNAL RELATIONSHIPS WITHIN LITHODIDAE

A monophyletic origin of lithodid crabs has been confirmed in several studies, both using molecular and morphological data (e.g. McLaughlin *et al.*, 2007; Reimann *et al.*, 2011; Bracken-Grissom *et al.*, 2013). Recent molecular studies, however, have left the status of the two lithodid subfamilies, Hapalogastrinae and Lithodinae, ambiguous (Hall & Thatje, 2009b; Bracken-Grissom *et al.*, 2013; Thatje & Hall, 2016). The two taxa are separated based on the presence of a calcified or uncalcified pleon (McLaughlin, 2014). Hapalogastrinae are mostly small-sized crabs with a soft, uncalcified pleon, and have been suggested to represent a morphological intermediate form between pagurid hermit crabs and the large-sized king crabs, most closely
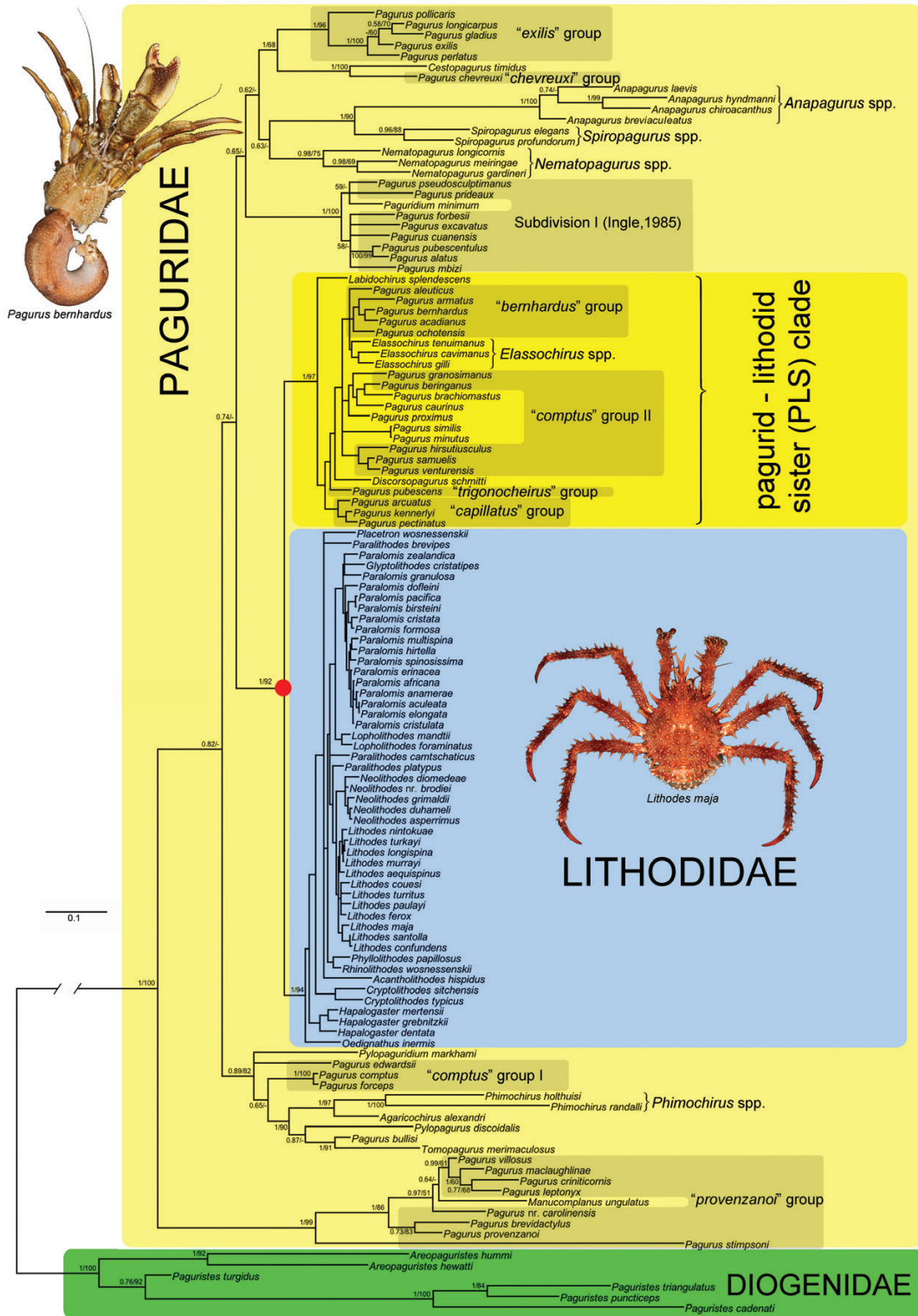
Figure 1. Bayesian 50% majority-rule tree of Paguridae, Lithodidae and Diogenidae (outgroup) for the five-gene concatenated data set. Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values over 50% are given on the nodes, respectively. Colour fields indicating Lithodidae (blue), Paguridae (yellow) and Diogenidae (green). The red dot indicates the node corresponding to the LCA shared by Lithodidae and pagurid hermit crabs. *Pagurus* species assigned to informal species groups are highlighted with a grey overlay.
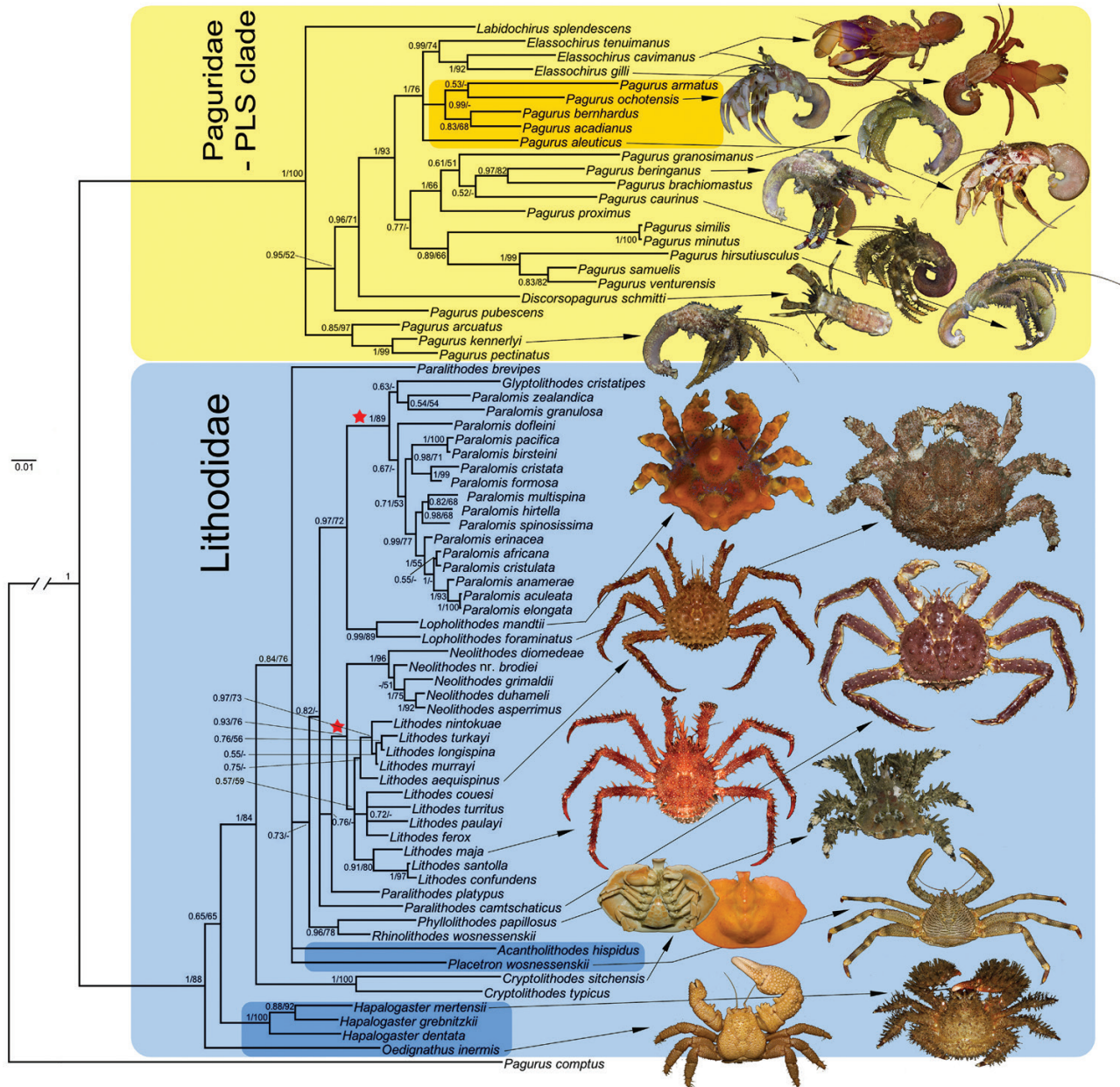
Figure 2. Bayesian 50% majority-rule tree of Lithodidae (blue) and the hermit crab clade, which constitutes the closest relatives within the Paguridae, the 'pagurid–lithodid sister clade' (PLS clade) (yellow) for the five-gene concatenated data set. Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values over 50% are given on the nodes, respectively. Species of the subfamily Hapalogastrinae are highlighted by dark blue overlay. *Pagurus s.s.* species of the '*bernhardus*' group are highlighted by dark yellow overlay. Red stars indicating clades with deep-sea radiations outside the North Pacific. Illustrated specimens are not to scale.

resembling the lithodid stem species (Bouvier, 1895; Boas, 1924; Richter & Scholtz, 1994; Keiler *et al.*, 2015). Recently, detailed morphological examinations of *Hapalogaster mertensii* Brandt, 1850 showed that this small lithodid crab possesses anatomical features of both Paguridae and Lithodidae. Most strikingly, *Hapalogaster* exhibits an uncalcified sac-like pleon

and also features characters of both groups in the vascular system (Keiler *et al.*, 2015). Morphological studies have supported the separation of lithodid crabs into the two distinct subfamilies. Hapalogastrinae were, however, only represented by the single genus *Hapalogaster* in these studies (Richter & Scholtz, 1994; Keiler & Richter, 2011; Keiler *et al.*, 2013). Few

previous molecular studies included representatives of Hapalogastrinae. In two studies, only one representative of each of Hapalogastrinae and Lithodinae was included (Ahyong *et al.*, 2009; Schnabel *et al.*, 2011). Two species of Hapalogastrinae were included in other studies (Hall & Thatje, 2009b; Bracken-Grissom *et al.*, 2013), together with a number of lithodine species. Hall & Thatje (2009a) found two monophyletic subfamilies, but with only low support. Bracken-Grissom *et al.* (2013) found different internal relationships within the Lithodidae, depending on whether only molecular data were used, or if the data set was combined with morphological data. In the combined data set, *Hapalogaster* and *Oedignathus* were resolved as basal within the Lithodidae. In analyses using molecular data alone on the other hand, *Oedignathus* was placed among the lithodid crabs and distant from *Hapalogaster*. This placement of *Oedignathus*, however, appears to be due to a number of apparent sequencing errors in the included 18S gene from this species (GenBank: Z14062) (Kim, Min & Kim, 1992), which we found to be highly conserved in Lithodidae. For this reason, this sequence was excluded from our analyses. Instead of a clear separation into Hapalogastrinae and Lithodinae, various small-sized, shallow-water taxa appear basal in the phylogenetic tree of Lithodidae from our analysis (Fig. 2). The Hapalogastrinae genera *Hapalogaster* and *Oedignathus* are resolved as basal to all other lithodids. Two other genera of Hapalogastrinae (*Placetron* and *Acantholithodes*) had not previously been included in any phylogenetic study. These were found nested within Lithodinae and appear more derived than the small-sized lithodine genus *Cryptolithodes*, rendering both subfamilies non-monophyletic. The basal position of some genera of Hapalogastrinae, as indicated by previous studies (Richter & Scholtz, 1994; Keiler & Richter, 2011; Keiler *et al.*, 2013), is in agreement with our results. A soft pleon, as a remnant of the hermit crab origin, must be considered as the plesiomorphic state of Lithodidae. The phylogenetic position of the small-sized lithodine genus *Cryptolithodes*, however, which is throughout heavily calcified, indicates that calcification of the pleon evolved at least twice within Lithodidae.

THE CLOSEST EXTANT RELATIVES TO LITHODIDAE

Our molecular analyses clearly show that a distinct, species-rich clade of hermit crabs forms the direct sister group to Lithodidae within the Paguridae (Fig. 1). Such a morphologically diverse and species-rich hermit crab sister clade to the lithodids is not as surprising as it might appear at first glance, given the species richness and morphological diversity of lithodid crabs themselves.

Previous molecular phylogenetic studies have suggested different pagurid taxa as the closest relatives to the king crabs, depending on the representation of pagurid species in the analyses. With internal relationships of the Paguridae largely unknown, especially regarding the polyphyletic genus *Pagurus*, the topology of previous phylogenetic studies depended on which *Pagurus* species were included in the data sets. The first molecular study on king crabs (Cunningham *et al.*, 1992) found the closest sister taxa to the lithodids to be a clade containing two species of the genus *Pagurus* [*Pagurus bernhardus* (Linnaeus, 1758) and *Pagurus acadianus* Benedict, 1901], together with the genera *Labidochirus* and *Elassochirus*. Two other *Pagurus* species included in the analyses were found to be more distantly related. Cunningham *et al.* highlighted the nested position of lithodids within the genus *Pagurus*. Richter & Scholtz (1994) subsequently noted that strong similarities of the first antennae between *P. bernhardus* and lithodid crabs support the results of Cunningham *et al.* (1992). Our analyses are in accordance with the finding of this first molecular study, as species resolved as closest relatives are also found in our PLS clade. Boas (1880b) initially assumed lithodids to be derived from the former *Eupagurus* Brandt, 1851, which contained some species of the genus *Pagurus*. Bouvier (1895) assumed lithodids as derived from an ancestor in which females have paired first pleopods, as present in the genus *Pylopagurus*. However, this taxon has since undergone major taxonomic revisions (McLaughlin, 1981; Lemaitre & McLaughlin, 2003), and it is thus unclear to which species Bouvier actually referred (Reimann *et al.*, 2011). Boas (1924) later suggested *Pylopagurus* and *Nematopagurus* as candidates for the closest relatives to lithodids. Also in a cladistic analysis based on foregut morphology, *Nematopagurus* and *Pylopagurus s.s.* were resolved as sister group to lithodids within other Paguridae (Reimann *et al.*, 2011), suggesting the same position to lithodids as assumed by Boas (1924). Our data, however, clearly show that neither *Nematopagurus*, *Pylopagurus*, nor any of the other genera of the '*Pylopagurus–Tomopagurus*' group after Lemaitre & McLaughlin (2003) included in our analyses are particularly closely related to the Lithodidae (Fig. 1). The occurrence of first pleopods in different Paguridae appears to be plesiomorphic (Richter & Scholtz, 1994) and might not have been present in the LCA of hermit crabs and lithodids. Another genus of hermit crabs, *Discorsopagurus*, came into focus as possibly the closest relative to lithodids after being included in a molecular phylogeny by Morrison *et al.* (2002). In addition to *Discorsopagurus schmitti* (Stevens, 1925), *Pagurus longicarpus* Say, 1817 and the lithodid

Cryptolithodes typicus Brandt, 1848 were included in this study. *Discorsopagurus* and *Cryptolithodes* showed a sister relationship, while *P. longicarpus* was found one node lower in the tree. This result might have initiated a focus on *Discorsopagurus* as a possible closest relative to lithodids, as it appeared more closely related than *Pagurus*. However, the reason that *Discorsopagurus* was resolved as a closer relative than a representative of *Pagurus* is merely due to the fact that *Discorsopagurus* is included in the PLS clade, while *P. longicarpus* is not found in this group (Fig. 1). Later studies confirmed a close relationship of *Discorsopagurus* to Lithodidae (Ahyong *et al.*, 2009; Schnabel *et al.*, 2011). Bracken-Grissom *et al.* (2013) included a larger number of Paguridae in their analyses and also here species of our PLS clade [*Labidochirus splendescens* (Owen, 1839), *D. schmitti* and *P. bernhardus*] were resolved with an equal sister relationship to lithodids based on molecular data alone. The authors, however, focused their discussion on the apparently closer relationship of *Discorsopagurus*, which was found when morphological data were added to the analyses. Based on their phylogenetic results, they suggested a *Discorsopagurus*-like hermit crab as the precursor to lithodids, which appeared plausible considering the North Pacific distribution of the genus (the region where lithodids are assumed to have originated; Hall & Thatje, 2009b). *Discorsopagurus* inhabits non-coiled housings, like polychaete tubes, and possesses an almost symmetrical pleon, with asymmetry restricted to the pleopods (Gherardi, 1996; Komai, 2003). Our data show that while *Discorsopagurus* is included in the PLS clade and thus shares the LCA to lithodid crabs within the Paguridae, it is not more closely related than other members of this clade with more typical pagurid morphologies. This indicates that the secondary pleon symmetry in *Discorsopagurus* and male lithodid crabs evolved independently and was unlikely present in the LCA.

Interestingly, no extant hermit crabs with tendencies towards a crab-like body shape have been suggested as precursor to lithodid crabs. Two species that show signs of carcinization from a typical pagurid morphology (Blackstone, 1989; Anker & Paulay, 2013) are found inside the PLS clade: *L. splendescens* and *Pagurus hirsutiusculus* (Dana, 1851) (Blackstone, 1985; Cunningham *et al.*, 1992). *Labidochirus*, a hermit crab with a fully calcified carapace, has been referred to as resembling a 'missing link' between hermit crabs and lithodid crabs (Jensen, 1995; Seeb *et al.*, 2002). The large number of conventional pagurid hermit crabs in the PLS clade, however, points to a LCA with a rather typical hermit crab morphology.

POLYPHYLY OF THE GENUS *PAGURUS*

The finding that Lithodidae are not only nested within the pagurid hermit crabs, but even inside the genus *Pagurus* (Cunningham *et al.*, 1992), caused much attention and disputes (McLaughlin *et al.*, 2004; Lemaitre & McLaughlin, 2009). The genus *Pagurus* was originally established by Fabricius (1775) as a heterogeneous group of non-crab-like species of Linnaeus' genus *Cancer* Linnaeus, 1758. A large range of hermit crabs was initially included in *Pagurus* and later assigned to new genera (McLaughlin, 1974). Our phylogenetic analyses show that today *Pagurus* is still highly polyphyletic. The species assigned to *Pagurus* do not possess unique morphological features, but rather display a 'standard' pagurid body plan (McLaughlin, 2003). The genus has been grouped into several informal morphological species groups (Forest & de Saint Laurent, 1968; McLaughlin, 1974; Lemaitre, McLaughlin & García-Gómez, 1982; Ingle, 1985; Lemaitre & Cruz-Castaño, 2004). In our phylogenetic tree, species of the genus *Pagurus* are divided into numerous distinct genetic lineages, often confirming previously recognized morpho-groups, for example the '*exilis*', '*bernhardus*', '*capillatus*' and '*provenzanoi*' groups, and subdivision I by Ingle (1985) (Fig. 1). Only representatives of the informal '*comptus*' group are found in two very distinct clades within the phylogenetic tree. However, due to the deviation from the group diagnostic characters in North Pacific representatives of this group, the '*comptus*' group has been highlighted as likely polyphyletic (McLaughlin, 1974).

McLaughlin (1974) suggests that *Pagurus* '*s.s.*' will eventually be restricted to a few species typified by *P. bernhardus,* which was selected as type species for the genus by Latreille (1810). Our phylogenetic analyses support this prediction, as a group of only few morphologically very similar species form a clade with *P. bernhardus*, without rendering the genus polyphyletic (Fig. 2). The species in this *Pagurus* '*s.s.*' group are the Northeastern Atlantic *P. bernhardus*; the Northwestern Atlantic *P. acadianus*; and the North Pacific *Pagurus aleuticus* (Benedict, 1892), *Pagurus armatus* (Dana, 1851) and *Pagurus ochotensis* Brandt, 1851, which have been grouped together in the '*bernhardus*' group (McLaughlin, 1974). Included in this '*bernhardus*' group are also three other species from the North Pacific (Komai, 1998; McLaughlin & Asakura, 2003; Lemaitre & Watabe, 2005), which were not included in our analyses. Of all the ~180 species currently assigned to *Pagurus* (Türkay, 2016), likely only these eight species can be included in the genus without rendering it polyphyletic.

TAXONOMIC HIERARCHY OF THE LITHODID CRABS

The deeply nested position of lithodid crabs within the hermit crab family Paguridae makes a phylogenetic classification difficult to apply. To further complicate issues, McLaughlin *et al.* (2007) proposed the taxonomic elevation of lithodid crabs to superfamily level, Lithodoidea, since the authors did not agree with the concept of a pagurid ancestry of the taxon. The two subfamilies of lithodid crabs were accordingly elevated from subfamily to family rankings: Lithodidae and Hapalogastridae. However, the phylogenetic position of lithodids outside the Paguroidea (McLaughlin *et al.*, 2007) contradicted all molecular and many morphological studies. The placement of a superfamily, Lithodoidea, within the family Paguridae subsequently has been highlighted as problematic, since it obscures evolutionary relationships (Ahyong *et al.*, 2009; Keiler *et al.*, 2013, 2015; Anker & Paulay, 2013). Further highlighting the problem caused by the elevation of the taxon is that Lithodidae *sensu* McLaughlin *et al.* (2007) is exclusive of hapalogastrids, while these were previously recognized as part of this family via the subfamily Hapalogastrinae.

The concept of a 'Lithodoidea' has subsequently only been used by a few authors (Ahyong *et al.*, 2009; De Grave *et al.*, 2009; Schnabel *et al.*, 2011; Tsang *et al.*, 2011; Bracken-Grissom *et al.*, 2013). Some authors adopted the two distinct family rankings (Lithodidae and Hapalogastridae) while rejecting the superfamily Lithodoidea to combine the two taxa (Keiler *et al.*, 2013, 2015). Others used the family Lithodidae in its former sense, containing the two subfamilies Lithodinae and Hapalogastrinae (Guzmán, 2009; Hall & Thatje, 2009b; Macpherson & Wehrtmann, 2010; Anker & Paulay, 2013), a system which we also use in this study. However, even the nested position of a family (Lithodidae) within another family (Paguridae) masks the true relationships of the groups. The fact that lithodids, in addition, appear nested within the polyphyletic genus *Pagurus* highlights this problem. Both Hapalogastrinae and Lithodinae appear non-monophyletic in our analyses. We, therefore, suggest a rather opposite taxonomic ranking to the one proposed by McLaughlin *et al.* (2007), by combining all lithodid crabs in a single taxon, and recognize its position within Paguridae by using the rank of a subfamily, Lithodinae. Hermit crabs within Paguridae, subsequently categorized under Pagurinae, are, however, still paraphyletic under this ranking. Pagurid hermit crabs are in need of an extensive taxonomic revision, which will need in-depth morphological and molecular investigations.

GEOGRAPHIC ORIGIN OF THE LITHODIDAE

Our finding that the closest hermit crab relatives to lithodid crabs predominantly consist of North Pacific species adds further support to a Northern Pacific origin of lithodids, as it suggests that the split between the PLS lineage and lithodids also occurred here. The four species of the PLS clade with a North Atlantic distribution have closely allied species in the Pacific: *P. bernhardus* from the North East Atlantic and *P. acadianus* from the North West Atlantic are sister species, with closely related species in the North Pacific, forming the '*bernhardus*' group (Fig. 2). *Pagurus pubescens* Krøyer, 1838, found on both sides of the North Atlantic, has closely allied species in the North Pacific, forming the '*trigonocheirus*' group. *Pagurus arcuatus* Squires, 1964, from the North West Atlantic, has closely allied species in the North Pacific, forming the '*capillatus*' group (McLaughlin, 1974). The terminal nodes in the phylogenetic tree leading to these Atlantic species show that their predecessors, one for the representatives of each group, must independently have entered the Atlantic via the Bering Strait.

The evolution of the deep-sea lineages followed a diversification of the taxon in the shallow North Pacific before changes in larval biology enabled certain taxa to extend their distribution into the deep sea (Hall & Thatje, 2009b; Thatje & Hall, 2016). Our data confirm the distribution of basal taxa of Lithodidae in the North Pacific and show two clear independent events of deep-sea radiation (Fig. 2). One event for *Paralomis*, including the monotypic *Glyptolithodes* which is found to be nested within the otherwise monophyletic genus *Paralomis* (Hall & Thatje, 2010), and one for *Lithodes* and *Neolithodes* (Fig. 2), of which *Neolithodes* reaches abyssal depths (Hall & Thatje, 2009b). Confining temperature boundaries have allowed only a few species from the boreal regions to re-emerge from the deep-sea into shallow-water habitats (Hall & Thatje, 2009b).

AGE OF THE ORIGIN OF THE LITHODIDAE

The origin of Lithodidae has been estimated from 15 to 13 Mya (mid to lower Miocene) based on molecular clock analyses of the mitochondrial 16S rRNA (Cunningham *et al.*, 1992). Following this study, the first lithodid crab known from the fossil record was described. This fossil species, *Paralomis debodeorum* Feldmann, 1998, has been dated to the mid to late Miocene of New Zealand and documents the presence of king crabs to at least 10 Mya in the South Pacific (Feldmann, 1998). In this context, the estimated lithodid origin obtained by Cunningham *et al.* (1992) appears quite recent. A slightly older origin, between 29 and 18 Mya, has been estimated using multiple genes, with fossil calibrations from the entire Anomura

(Bracken-Grissom *et al.*, 2013). Molecular clock analyses might, however, be influenced by a low sequence diversity within the Lithodidae (Snow, 2010; Matzen da Silva *et al.*, 2011b). On the other hand, the deposit from which *P. debodeorum* has been discovered is difficult to date precisely (Feldmann, 1998; Feldmann, Schweitzer & McLauchlan, 2006), leaving a relatively recent radiation, reflected in low genetic variation, as a possibility. A low sequence divergence within Lithodidae was also found in our own sequence data, especially for the nuclear rRNA subunits. The entire ~1800 bp long 18S fragment only showed minimal variation, and even sequences obtained from different lithodid genera were found to be identical. The taxonomic assignment of fossil hermit crabs is problematic, and 'lump genera' such as *Palaeopagurus* Van Straelen, 1924 or *Pagurus* have been used for most species (Jagt *et al.*, 2006). The highly polyphyletic pattern of extant species of *Pagurus*, as shown in our phylogeny (Fig. 1), further highlights this problem. Accurate divergence timing using fossil calibrations for the Paguridae is, therefore, problematic. Detailed investigation of phylogenetic relationships within various Paguridae taxa, and the species-rich deep-sea lithodid genera, in conjunction with biogeography might eventually provide a more reliable divergence estimate through the timing of geological events.

EVOLUTIONARY SCENARIOS FOR THE LITHODIDAE

Taxa within Lithodidae that are found basal in our phylogenetic analyses inhabit shallow-water, rocky habitats, which is in agreement with previous studies that suggested a shallow-water origin of lithodids (Makarov, 1938; Zaklan, 2001; Hall & Thatje, 2009b). Our finding that the closest hermit crab relatives also inhabit shallow habitats further supports this theory. This habitat must have played a key factor in the process of changing from a shell-utilizing to a free-living lifestyle. Crab-like forms appear to have evolved multiple times in shallow-water habitats (Morrison *et al.*, 2002; Tsang *et al.*, 2011), and these independent transitions offer strong evidence for the adaptive advantages of the crab-like form in relation to habitat type (Tsang *et al.*, 2011). Most anomurans with a crab-like morphology are found living in hard bottom habitats, under boulders and stones, where a short, compact pleon is advantageous in exploring crevices (Tsang *et al.*, 2011). Carcinization in hermit crabs is more complex than in other decapods, since this process, besides the broadening of the cephalothorax and reduction and underfolding of the pleon, also implies reorganization and calcification of the cephalothorax and pleon as these animals abandon the use of domiciles (Anker & Paulay, 2013). Besides the lithodids,

tendencies towards carcinization, involving reduction or armouring of the pleon, tendency to lose domiciles and calcification of the cephalothorax, occurred independently in several groups of hermit crabs (Anker & Paulay, 2013). The abandonment or reduced use of a portable domicile must be seen as the most important step towards this morphological transformation. Different alternative pathways of leaving a protective housing are possible, and multiple scenarios for the cause of the predecessor of lithodid crabs leaving a protective shell exist. The various degrees of carcinization present in different hermit crabs give insights into the possibilities for morphological transition, but none of these taxa represent direct evolutionary intermediate forms between hermit crabs and lithodid crabs. Cunningham *et al.* (1992) explained carcinization in king crabs via a heterochronic shift in developmental timing, the extension of the ancestral hermit crab ontogeny to produce a carcinized adult, termed peramorphosis. In this scenario, ancestral hermit crab allometries were modified to accommodate an extended ontogeny and larger body size. In particular, Cunningham *et al.* (1992) highlighted the terrestrial hermit crab *Birgus latro* (Linnaeus, 1767), which, having a normal hermit crab habitus as a juvenile, outgrows its protective shell during ontogeny and develops a crab-like, calcified body (Greenaway, 2003). In Lithodidae, a crab-like morphology is, however, already apparent at metamorphosis (Morrison *et al.*, 2002; McLaughlin *et al.*, 2004), and Morrison *et al.* (2002) suggested a somewhat different mechanism of heterochronic shift, in the form of displacement heterochrony (Alberch *et al.*, 1979). The size of the largest available gastropod shell limits the size of hermit crabs (Cunningham *et al.*, 1992), and a lack of suitable shells has been discussed as a factor in the carcinization of lithodids (Richter & Scholtz, 1994). An absence of sufficiently large shells, due to an increase in size in the ancestral lineage of Lithodidae, leading to limited resources of suitable housings was rejected, since many lithodid species, in particular Hapalogastrinae, are not very large. As a more likely alternative, the lack of suitable shells in certain habitats was suggested as a possible starting point for lithodid evolution (Richter & Scholtz, 1994). Our results also point to a small-sized LCA, making the limitation of large shells as causation behind the evolution of lithodids unlikely. Some species of hermit crabs with tendencies towards carcinization are restricted by the availability of large gastropod shells, for example due to a deep-sea habitat, such as *Porcellanopagurus* Filhol, 1885; *Solitariopagurus* Türkay, 1986; and *Patagurus* Anker & Paulay, 2013 (McLaughlin & Lemaitre, 1997; Anker & Paulay, 2013). In some of these taxa, the pleon is reduced in size and only covered by a shell, which is

too small for the animal to retract into (McLaughlin & Lemaitre, 1997; Anker & Paulay, 2013). From the early Miocene, large gastropod species were, however, never rare in the shallow North Pacific (Vermeij, 2012), and also the parallel diversification of hermit crabs in the same region, as indicated from our phylogeny, further points to a scenario without a general lack of gastropod shells. Furthermore, the large increase in body size of some lithodid taxa clearly occurred after the acquisition of the crab-like form, as basal taxa within the Lithodidae are of only moderate size.

ADVANTAGES OF ABANDONING OF A DOMICILE

While the disadvantages of abandoning a protective housing and subsequently exposing the soft pleon have been highlighted as a maladaptive evolutionary scenario (McLaughlin & Lemaitre, 1997; McLaughlin *et al.*, 2004), becoming independent from a housing also brings clear advantages. Competition for housings, and the need to find and change suitable housings during ontogeny are probably the most obvious ones. Inhabiting a gastropod shell, however, also requires a heavy object to be carried, greatly reducing mobility. Leaving the constraint of being bound to a foreign shell results in an increase in agility and speed, potentially making new prey sources available and enabling escape from predators (Blackstone, 1989; Anker & Paulay, 2013). The advantages of higher mobility also include the possibility of inhabiting new microhabitats precluded by carrying a bulky and heavy shell, like crevices or rock overhangs. As basal lithodids are found in such habitats today (Jensen, 1995), the enhanced mobility resulting from abandoning a protective housing is likely a key factor behind the evolutionary pathway of Lithodidae. An example of enhanced mobility by reducing the weight of a protective housing is found in the intertidal hermit crab *P. hirsutiusculus*, which uses only small shells in which the animal cannot fully retract. *Pagurus hirsutiusculus* shows tendencies towards carcinization, such as a broadened carapace and stronger armature of the pleon (McLaughlin, 1974). This species is very agile and often abandons its housing in escape reactions (Blackstone, 1989). A higher level of activity permits the animal to rely on speed of escape, rather than a housing for protection, which could favour shell loss and carcinization (Blackstone, 1989). This example illustrates how slight changes in ecology and shell-use might lead to carcinization in hermit crabs, without a restriction of housings. A number of hermit crab species have obligate commensal relationships with certain species of actinarians (Williams & McDermott, 2004). In the most advanced of these symbiotic relationships, the sea anemone builds the entire housing for the crab or greatly enlarges an originally present small gastropod shell. This lightweight housing protects its inhabitant not by a heavily calcified structure, but by a soft housing with protrudable thread-like acontias, which are loaded with poisonous nematocysts for defence. An increase in mobility, via reducing the weight of the shelter, is also an advantage of these relationships. The pagurid *L. splendescens*, which is found in the PLS clade, has such a symbiotic relationship. The lightweight housing, together with long walking legs for rapid locomotion, gives the animal a much higher mobility than seen in conventional hermit crabs. *Labidochirus splendescens* also shows tendency towards carcinization, like a broadened, fully calcified carapace, and an only moderate-sized pleon (McLaughlin & Lemaitre, 1997; Anker & Paulay, 2013).

CONCLUSIONS

The Lithodidae are deeply nested within the hermit crab family Paguridae and show a clear sister relationship with a clade of predominantly North Pacific shallow-water hermit crabs. Lithodid crabs are even found nested within a highly polyphyletic hermit crab genus *Pagurus*, confirming the results of the very first molecular study on the taxon by Cunningham *et al.* (1992). The stem species of Lithodidae inhabited shallow waters of the North Pacific with no general shortage of suitable gastropod shells. A crab-like morphology likely evolved gradually due to the adoption of smaller housings for the benefit of higher mobility. The basal position of small-sized taxa clearly indicates that an increase in body size was not the trigger for developing a crab-like habitus in the Lithodidae. The abandonment of a domicile, however, enabled the development of gigantism in lithodid crabs, since available gastropod shells for housing no longer set a size limitation. Enhanced armour in the form of spines and calcification, and an increase in size, enabled king crabs to leave the initial protective environment and expand into non-sheltered habitats. In the deep sea, the taxon could finally diversify on a global scale.

Knowledge about phylogenetic relationships within the diverse deep-sea genera is still fragmentary. However, the species richness of these genera might eventually enable a detailed reconstruction of the dispersal routes within the deep-sea lineages of king crabs.

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