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Decapod Crustacean Phylogenetics

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Phylogeny of Marine Clawed Lobster Families Nephropidae Dana, 1852, and Thaumastochelidae Bate, 1888, Based on Mitochondrial Genes

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ABSTRACT

Phylogenetic relationships of extant marine clawed lobsters of the families Nephropidae and Thaumastochelidae were analyzed based on partial sequences of the 12S and 16S mitochondrial rRNA genes. The ingroup sample consisted of 17 species and ten genera of the Nephropidae as well as two species and two genera of the Thaumastochelidae. The family Enoplometopidae was used as an outgroup. A total of 875 base pairs, with 241 parsimony informative sites, was analyzed. Bayesian (MRBAYES) and maximum likelihood (PAUP) analyses produced similar topologies. The ML tree was well supported at most nodes. Generic monophyly was confirmed for all five genera represented by two or more species. *Acanthacaris* is the least derived among genera included in the analysis. It was resolved as a sister taxon to all other nephropids (including thaumastochelids). The thaumastochelids are monophyletic but nested within Nephropidae; thus, family-level status for thaumastochelids was not supported. Some nephropid genera, previously regarded as close relatives on a morphological basis (e.g., *Homarus* and *Homarinus*, or *Nephrops* and *Metanephrops*), instead appear to be cases of morphological convergence.

1 INTRODUCTION

1.1 *General*

Marine clawed lobsters include the families Erymidae van Straelen, 1924 (Lower Triassic–Upper Cretaceous), Chimerastacidae Amatie et al., 2004 (Middle Triassic), Chilenophoberidae Tshudy & Babcock, 1997 (Middle Jurassic–Lower Cretaceous), Nephropidae Dana, 1852 (Lower Cretaceous–Recent), Thaumastochelidae Bate, 1888 (Upper Cretaceous–Recent), and Enoplometopidae de Saint Laurent, 1988 (Recent). The family Nephropidae is the most diverse, consisting of 14 genera (11 extant [*Acanthacaris* Bate, 1888; *Eunephrops* Smith, 1885; *Homarinus* Kornfield et al., 1995; *Homarus* Weber, 1795; *Metanephrops* Jenkins, 1972; *Nephropides* Manning, 1969; *Nephrops* Leach, 1814; *Nephropsis* Wood-Mason, 1873; *Thymopides* Burukovsky & Averin, 1976; *Thymops* Holthuis, 1974; *Thymopsis* Holthuis, 1974] and three extinct [*Hoploparia* McCoy, 1849; *Jagtia* Tshudy &

Sorhannus, 2000; *Palaeonephrops* Mertin, 1941]). The present study investigates phylogenetic relationships of the clawed lobster genera of the families Nephropidae and Thaumastochelidae.

Phylogeny of the clawed lobsters is of interest for more than their intrinsic generic relationships. It potentially provides insights into questions of general biological and paleontological interest such as rates of morphological and molecular evolution, or the frequency and distribution of molecular or morphologic homoplasy. Likewise, of general interest is the comparison of phylogenies produced by different methods, including traditional intuitive schemes versus cladistic analyses, and morphology- versus DNA-based cladistic analyses. Clawed lobsters, by virtue of their complex morphology, long range in the fossil record, wide geographic range, and ecological diversity, are a group well suited for such investigations.

1.2 *Previous work, morphological and molecular*

A number of workers have conducted morphology-based cladistic analyses on clawed lobsters (Tshudy 1993 [20 genera]; Williams 1995 [four genera]; Tshudy & Babcock 1997 [22 genera]; Tshudy & Sorhannus 2000a [19 genera], 2000b [13 genera]; Dixon et al. 2003 [four genera]; Rode & Babcock 2003 [nine genera]; Ahyong & O'Meally 2004 [five genera]; Amati et al. 2004 [seven genera]; Ahyong 2006 [26 genera]. Ahyong (2006) included all (14) nephropid and (three) thaumastochelid genera, fossil and extant, in the largest matrix published to date. Ahyong's (2006) character matrix is similar to earlier matrices of Tshudy (1993) and Tshudy & Babcock (1997), and thus does not constitute a robust test of those trees. Nonetheless, Ahyong (2006) added additional characters and included for the first time taxa such as *Neoglyphea* Forest & de Saint Laurent, 1975, *Enoplometopus* A. Milne-Edwards, 1862, and the Uncinidae Beurlen, 1928.

Few workers have conducted DNA-based cladistic analyses on the clawed lobsters. Tam & Kornfield (1998), using 16S mtDNA, produced a tree including five nephropid genera (*Homarus*, *Homarinus*, *Metanephrops*, *Nephrops*, *Nephropsis*). Ahyong & O'Meally (2004) used 16S mtDNA along with 18S and 28S nuclear DNA data (2,500 bp total) to evaluate reptant decapod phylogeny, including six lobster genera (*Enoplometopus*, *Homarus*, *Metanephrops*, *Neoglyphea*, *Nephropsis*, and *Thaumastochelopsis* Bruce, 1988). Porter et al. (2005) used 16S mtDNA along with 18S and 28S nuclear DNA data and the histone H3 gene (3,601 bp total) to evaluate decapod phylogeny (43 genera), including four lobster genera (*Acanthacaris*, *Homarus*, *Nephrops*, and *Nephropsis*). Chu et al. (2006) produced a 12S mtDNA-based tree for ten clawed lobster genera using *Neoglyphea* as an outgroup. The present study concerns the phylogenetic relationships of the Recent clawed lobster genera of the Nephropidae and Thaumastochelidae. Our analysis is based on partial sequences of mitochondrial 12S and 16S genes and includes 12 ingroup genera (adding *Homarinus*, *Thaumastochelopsis*, and *Thymops* to those analyzed by Chu et al. 2006).

2 MATERIALS AND METHODS

2.1 *Taxon sampling*

The ingroup (Table 1) consists of 21 terminals representing 17 species and ten genera of the Nephropidae as well as two species and two genera of the Thaumastochelidae. The family Thaumastochelidae was included in the analysis because family-level status has been debated and remains equivocal. In some studies, members of this family have been suggested to constitute their own family (Holthuis 1974; Tshudy & Sorhannus 2000a, b; Dixon et al. 2003; Schram & Dixon 2004; Ahyong & O'Meally 2004; Ahyong 2006), whereas other studies include them as part of Nephropidae (Tshudy & Babcock 1997; Chu et al. 2006).

The outgroup used in our study was the family Enoplometopidae, recently found to be the sister group to the Nephropidae + Thaumastochelidae in morphological (Ahyong & O'Meally 2004;

Table 1. List of specimens for which 16S mtDNA and 12S mtDNA were sequenced. CBM = Natural History Museum and Institute, Chiba; CNCR = Colección Nacional de Crustceos, Instituto de Biologa, UNAM; EUPG = Edinboro University of Pennsylvania; MNHN = Muséum National d'Histoire Naturelle, Paris; NTM = Museum of Art Gallery of the Northern Territory, Darwin; NTOU = National Taiwan Ocean University; USNM = National Museum of Natural History, Smithsonian Institution, Washington, D.C.; 1 = Aquarium shop, origin unknown; 2 = Supermarket, origin unknown.

Species	Catalog No.	Locality	GenBank Accession No. 12S	GenBank Accession No. 16S
Acanthacaris tenuimana	MNHN-As639	Solomon Islands	DQ298420	EU882871
Enoplometopus crosnieri	NTOU-M00602	Taiwan	DQ298423	EU882870
Enoplometopus daumi	NTOU-M00171	Singapore[1]	DQ298421	EU882868
Enoplometopus debelius	NTOU-00173	Singapore[1]	DQ298422	EU882869
Enoplometopus occidentalis	NTOU-M00152	Taiwan	DQ298424	EU882871
Eunephrops cadenasi	MNHN-As640	Guadeloupe	DQ298425	EU882873
Eunephrops manningi	MNHN-As641	Guadeloupe	DQ298426	EU882874
Homarinus capensis	USNM251453	S. Africa	EU882895	EU882887
Homarinus capensis	USNM251454	S. Africa	EU882896	EU882888
Homarus americanus	EUPGEO4001	U.S.A.	DQ298427	EU882875
Homarus gammarus	NTOU-M00819	France[2]	DQ298428	EU882876
Metanephrops japonicus	NTOU-M00521	Japan	EU882897	EU882889
Metanephrops rubellus	NTOU-M00074	Brazil	DQ298429	EU882877
Metanephrops thomsoni	NTOU-M00504	Taiwan	DQ298430	EU882878
Nephropides caribaeus	MNHN-As642	Guadeloupe	DQ298432	EU882879
Nephrops norvegicus	CBM-ZC7438	France[2]	DQ298433	EU882881
Nephropsis aculeata	CNCR21650	Mexico	EU882892	EU882884
Nephropsis aculeata	CNCR21660	Mexico	EU882893	EU882885
Nephropsis rosea	CNCR21631	Mexico	EU882894	EU882886
Nephropsis serrata	NTOU-M00157	Taiwan	DQ298434	EU882881
Nephropsis stewarti	NTOU-M00505	Taiwan	DQ298435	EU882882
Thaumastocheles japonicus	NTOU-M00168	Taiwan	DQ298438	EU882866
Thaumastochelopsis wardi	NTM-Cr.004231	Australia	EU882891	EU882867
Thymopides grobovi	MNHN-As181	Kerguelen Island	DQ298436	EU882883
Thymops birsteni	USNM291290	Chile	EU882898	EU882890

Ahyong 2006) and molecular analyses (Ahyong & O'Meally 2004; Tsang et al. 2008; Chu et al. this volume). The monogeneric Enoplometopidae is represented in the analysis by four species: *Enoplometopus crosnieri* Chan & Yu, 1998, *E. daumi* Holthuis, 1983, *E. debelius* Holthuis, 1983, and *E. occidentalis* (Randall, 1840).

2.2 *Tissue sampling*

Tissue samples used in this study were derived from freshly collected specimens or, more often, from preserved museum collections (Table 1). On collection, specimens were either frozen on site and later transferred to 70% ethyl alcohol (ETOH) or directly preserved in 70% ETOH. Species identification was based on morphology (Holthuis 1974, 1991; Tshudy 1993).

2.3 *DNA extraction*

DNA extraction, amplification, and sequencing were conducted at both the University of Louisiana Lafayette and the Chinese University of Hong Kong. Total genomic DNA was extracted from fresh or ethanol-fixed tissue samples collected from the abdomen (ventral side) or pereiopods. Muscle was ground and then incubated for 1–12 h in 600 μl of lysis buffer (100 mM EDTA, 10 mM tris pH 7.5, 1% SDS) at 65°C; protein was separated by addition of 200 μl 7.5 M of ammonium acetate and subsequent centrifugation. DNA was precipitated by addition of 600 μl of cold isopropanol followed by centrifugation; the resulting pellet was rinsed in 70% ETOH, dried in a speed vacuum system (DNA110 Speed Vac), and resuspended in 10–20 μl of TE buffer (10 mM TRIS, 1 mM EDTA). For samples extracted at the Chinese University of Hong Kong, total DNA was obtained from pleopod muscles (10–15 mg) with the QIAamp DNA Mini Kit (QIAGEN) following manufacturer's instructions. DNA was eluted in 200 μl of distilled water.

2.4 *DNA amplification and sequencing*

Two mitochondrial ribosomal genes, the 12S and 16S rRNA, were selected because of their proven utility in resolving generic relationships for other decapods (Kornfield et al. 1995; Schubart et al. 2000; Robles et al. 2007; Chan et al. 2008). Standard PCR amplification and automated sequencing protocols were used to sequence a fragment of approximately 400 bp of the 12S mtDNA and 550 bp of the 16S mtDNA. Both strands were sequenced for each gene. In all cases, the 12S and 16S sequences were derived from the same specimen. When possible, more than one species of each genus was included in our analysis.

Primers used for the 12S fragment were 12Sai (5'-AAA CTA GCA TTA GAT ACC CCT ATT AT-3') (Palumbi et al. 1991) and 12H2 (5'-ATG CAC TTT CCA GTA CAT CTA C-3') (Colbourne & Hebert 1996). Primers used for the 16S fragment were 16ar (5'-CGC CTG TTT ATC AAA AAC AT-3'), 16br (5'- CCG GTC TGA ACT CAG ATC ACG T-3') (Palumbi et al. 1991), 1472 (5'-AGA TAG AAA CCA ACC TGG-3') (Crandall & Fitzpatrick 1996), and 16L2 (5'-TGC CTG TTT ATC AAA AAC AT-3') (Schubart et al. 2002). Reactions were performed in 25 μl volumes (200 M each dntp, 1X buffer, 0.5 μM each primer, 1 unit Taq polymerase, 1 μl extracted DNA). Thermal cycling was performed as follows: initial denaturation for 10 min at 94–95°C followed by 40–42 cycles of 1 min at 94–95°C, 1–1:30 min at 48°C and 1:30–2 min at 72°C, with a final extension of 10 min at 72°C. PCR products were purified using 100,000 MW filters (Microcon-100® Millipore Corp.) and sequenced with the ABI BigDye® terminator mix (Applied Biosystems). Both PCR and cycle sequence reactions were conducted on a Robocycler® 96 cycler. Sequencing products were run on either a 310 or 3100 Applied Biosystems® automated sequencer.

2.5 *Sequence alignment and nucleotide composition*

Consensus of complementary sequences of the gene was obtained with the Sequencher® software program (ver 4.1, Genecodes, Ann Arbor, MI). Alignment of consensus sequences was performed with Clustal W, as implemented in Bioedit (Hall 1999) with the following settings: 6-2/6-2 penalty (opening-gap extension, pairwise/multiple alignment respectively). Base composition, pattern of substitution for pairwise comparison, and analysis of variability along both fragments of the 12S and the 16S mtDNA were analyzed in PAUP 4.0 beta 10 (Swofford 1993). Homogeneity of nucleotide frequency among taxa was also assessed for each gene with a χ^2 test as implemented in PAUP. The 12S and 16S data sets were combined for analysis. Partition homogeneity was assessed by the incongruence length difference (ILD) test as implemented in PAUP (Swofford 1993).

2.6 *Phylogenetic analysis*

Phylogenetic analyses were conducted using MRBAYES v.3.17 software for Bayesian analysis (BAY) and PAUP 4.0 beta 10 (Swofford 1993) for maximum likelihood (ML) analysis. Prior to conducting the BAY or ML analyses, the model of evolution that best fit the data was determined using MODELTEST v.3.7 (Posada & Crandall 1998). The Bayesian analysis was performed by sampling one tree every 100 generations for 1,000,000 generations, starting with a random tree, thus generating 10,001 trees. A preliminary analysis showed that stasis was reached at approximately 10,000 generations. Thus, we discarded 101 trees corresponding to those generations and obtained 50% majority rule consensus trees from the remaining 9,900 saved trees using PAUP. ML analysis was carried out with a distance correction set with the parameters obtained from MODELTEST (Posada & Crandall 1998). Analysis was performed as a heuristic search with gaps treated as missing data, multistate characters interpreted as uncertain, and all characters unordered. The search was conducted with a random sequence addition of taxa and tree bisection and reconnection as branch swapping option. Relative stability of clades under ML was determined from 100 bootstrap pseudoreplicates based on the same parameters as above. Bootstrap proportions >50% (for ML) and posterior probabilities (for BAY) are indicated in Figure 1.

3 RESULTS

3.1 *Nucleotide composition*

We produced 12S and 16S sequence data for 23 species (25 specimens) resulting in an alignment of 50 sequences. Sequences and alignments were submitted to GenBank as a PopSet. Our 12S alignment included a total of 407 bp of which 246 bp were constant, 33 were variable but not parsimony informative, and 128 characters were parsimony informative. The nucleotide composition of the database can be considered homogeneous ($\chi^2 = 27.293$, df = 72, P = 0.999) with a larger percentage of A–T (36.7%–37.0% respectively). Our 16S alignment included a total of 537 bp, of which 305 bp were constant, 65 were variable but parsimony uninformative, and 167 were parsimony informative. The nucleotide composition of the database can be considered homogeneous ($\chi^2 = 31.636$, df = 72, P = 0.999) with a larger percentage of A–T (32.8%–34.8% respectively). The combined alignment included 944 bp. We also excluded 69 saturated characters, 21 from the 12S fragment and 48 from the 16S fragment. From the remaining 875 characters, 544 were constant, 90 were variable but not parsimony informative, and 241 were parsimony informative. The ILD test showed no significant incongruence among gene segments (P = 0.462). Thus, all phylogenetic analyses were performed with a single data set including both genes.

3.2 *Phylogenetic analyses*

The best-fit model of nucleotide substitution, selected with the Akaike information criterion (AIC; Akaike 1974) as implemented in MODELTEST (Posada & Crandall 1998), was the HKY model (Hasegawa et al. 1985), with proportion of invariable sites (Γ) and a gamma distribution (δ), with the following parameters: assumed nucleotide frequencies: A = 0.3518, C = 0.0890, G = 0.1804, T = 0.3788; with transition/transversion ratio = 3.967; proportion of invariable sites Γ = 0.315; variable sites followed a gamma distribution with shape parameter δ = 0.498. These values were used for both ML and BAY analyses, which produced the same topology. We thus present a single tree obtained with ML analysis (ML score = 4986.170) that includes both ML bootstrap as well as Bayesian posterior probabilities (Fig. 1). In both analyses, monophyly of all five genera represented by two or more species received strong support values.

The ML tree based on the 12S and 16S genes is generally well supported at most, though not all, nodes (Fig. 1). Representative species of the putative family Thaumastochelidae were found

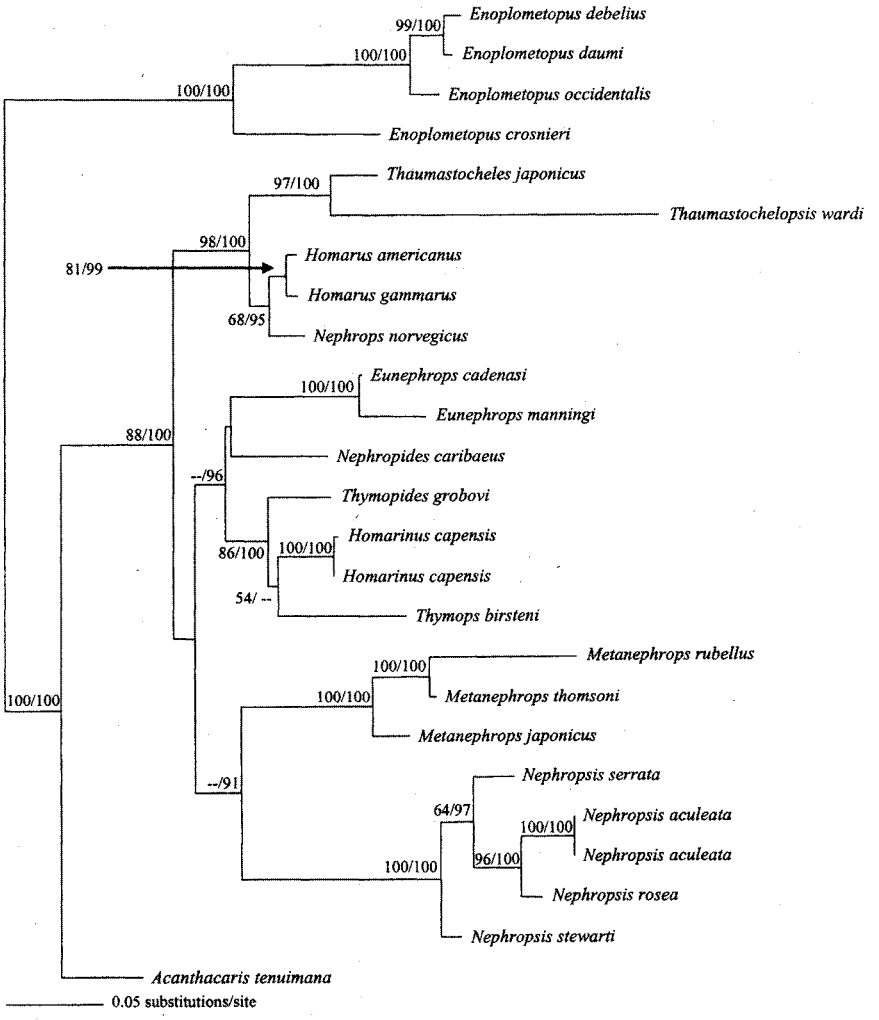

Figure 1. ML tree based on combined 16S and 12S sequences. ML bootstrap proportions (>50%) and Bayesian posterior probabilities indicated at nodes (ML/BAY).

to be monophyletic but nested within the Nephropidae (Fig. 1). *Acanthacaris* is the sister taxon to the remaining nephropids *sensu lato*. Among the latter, three clades were recovered. Relationships among these three clades cannot be considered resolved since they were not well supported by either bootstrap or Bayesian posterior probabilities. One clade included *Homarus*, *Nephrops*, *Thaumastocheles*, and *Thaumastochelopsis*. A second clade included the genera *Eunephrops*, *Nephropides*, *Thymopides*, *Homarinus*, and *Thymops*, although it was supported only by BAY. *Metanephrops* and *Nephropsis* formed a third clade, though it too was supported only by BAY.

4 DISCUSSION

To more fully understand relationships of the marine clawed lobsters, it is optimal to have a taxonomically comprehensive (all extant genera) molecular phylogenetic analysis based on multiple genes along with an equally comprehensive morphological study (all extant and extinct genera) based on a large data matrix. The present study analyzes two mitochondrial genes (12S and 16S) as a step toward this objective. While it would be ideal to root both the morphological and molecular

trees to the same outgroup, that is so far impractical. The most appropriate outgroup for the present DNA analysis, Enoplometopidae, has no fossil record, although potentially Uncinidae may be an enoplometopid (Ahyong 2006). Fortunately, in the case of the marine clawed lobsters, our unpublished DNA data indicate that ingroup topology is insensitive to a range of potential outgroups such as freshwater crayfish (*Astacus*, *Parastacus*, *Cambarus*), glypheoids (*Neoglyphea*), or Enoplometopidae.

4.1 *Comparison with previous works*

Our results for *Acanthacaris* corroborate those of Porter et al. (2005), who found good support for *Acanthacaris* as a sister taxon to the three remaining nephropid genera in their 43-genus analysis of decapod phylogeny. Topology of the *Nephropsis* + *Nephrops* + *Homarus* clade in Porter et al. (2005) is also consistent with our results. Topology of the present 12S–16S tree (12 genera) is nearly identical to the 12S tree (ten genera) of Chu et al. (2006), despite their using *Neoglyphea* as the outgroup. The topology of our 12S–16S tree differs somewhat from that of the 16S–18S–28S tree of Ahyong & O'Meally (2004), who included five genera of clawed lobsters (*Enoplometopus*, *Homarus*, *Metanephrops*, *Nephropsis*, *Thaumastochelopsis*) in their analysis of 45 decapod genera. The disagreement in topologies is in the arrangement of three nephropid genera: *Homarinus*, *Metanephrops*, and *Nephropsis*. Ahyong & O'Meally (2004), analyzing three nephropid genera, found *Nephropsis* to be the sister to *Metanephops* + *Homarus*. Our analysis shows *Metanephrops* and *Nephropsis* are closer to each other than either is to *Homarus*. However, in addition to their analysis encompassing a taxonomically broader group of decapod genera, they used a species of *Stenopus* Latreille, 1819, representing the Stenopodidae Claus, 1872 (consistently identified as sister group to reptantian decapods by Ahyong & O'Meally 2004), as their outgroup. Tam & Kornfield (1998) analyzed five nephropid genera using mitochondrial 16S rRNA and produced trees that, while not well resolved, show either *Nephropsis* (via maximum parsimony) or *Metanephrops* (via neighbor joining) as sister to the remaining nephropid genera analyzed.

4.2 Acanthacaris

Acanthacaris is determined here (Fig. 1), as in the multi-locus analysis of Porter et al. (2005) and the 12S analysis by Chu et al. (2006), to be the sister taxon to the remaining nephropoids. Most previous morphological studies (Tshudy & Babcock 1997; Tshudy & Sorhannus 2000a, b; Ahyong 2006) found *Acanthacaris* to be deeply nested within Nephropidae rather than the sister taxon to the remaining genera. This disagreement between morphological and molecular topologies is marked and is largely due to the many autapomorphies of *Acanthacaris* and unstable rooting of the morphological trees. In comparison to other nephropoid genera, *Acanthacaris* has many distinctive autapomorphies including: 1) a laterally compressed rostrum; 2) a single row of dorsal rostral spines; 3) parallel submedian carinae on the telson; 4) an extremely large scaphocerite extending almost to the end of the antennal peduncle; and 5) delicately constructed, symmetrical claws, each with a narrow, cylindrical palm and fingers bearing acicular denticles. However, these features, being unique, are cladistically uninformative. Thus, very few character states remain to robustly position *Acanthacaris* (irrespective of whether they are convergent). In addition, the position of the root, and thus *Acanthacaris*, in the morphological analysis is sensitive to outgroup choice (Tshudy et al., unpublished data). Significantly, however, morphological analyses, using an identical group of taxa, recover an identical position for *Acanthacaris* as sister to the remaining nephropids (Tshudy et al., unpublished data). In terms of branch support, the molecular data provide strongest support for the "basal" position of *Acanthacaris*, using a range of outgroups, so we may be justified in favoring the molecular results. Future morphological studies should closely reconsider the apparently

autapomorphous character states of *Acanthacaris* to determine whether, on closer inspection, they might be related to states in other taxa.

Acanthacaris is a blind, deep-sea (229–2161 m) lobster with no known close extant relatives and no known fossil relatives. *Palaeophoberus* Glaessner, 1932, previously thought to be related to *Acanthacaris* (Glaessner 1932, 1969; Mertin 1941; Burukovsky & Ckreko 1986), is now regarded as a chilenophoberid. At present, we cannot reliably infer whether the blind *Acanthacaris* evolved in the deep sea or, like the *Oncopareia-Thaumastocheles* lineage, lost its eyes through a migration from shallow, shelf depths into deeper, aphotic habitats.

4.3 *Status of Thaumastochelidae*

The family Thaumastochelidae is represented in this analysis by both of its Recent genera, *Thaumastocheles* and *Thaumastochelopsis*. These genera, along with the fossil (Late Cretaceous-Miocene) genus *Oncopareia* Bosquet, 1854, form a morphologically distinctive and cladistically cohesive group. The monophyly of the thaumastochelids has been supported by previous morphological studies (Tshudy & Babcock 1997; Tshudy & Sorhannus 2000a, b). Tshudy et al. (unpublished data), analyzing a 90-character morphology matrix, found the thaumastochelids united by three unambiguous synapomorphies: first pereiopod palm bulbous; telson wider than long; and uropodal endopod much smaller than exopod. Aside from these synapomorphies, all thaumastochelids have very distinctive abdominal pleura that are wider than long and quadrate, and even more distinctive first pereiopods with very long, slender fingers armed with acicular denticles. The close relationship among these three genera is undisputed, but their family-level status has been debated and has remained equivocal. Holthuis (1974) recognized the family, as did morphological cladistic analyses of Tshudy & Sorhannus (2000a, b), Dixon et al. (2003), Schram & Dixon (2004), Ahyong & O'Meally (2004), and Ahyong (2006). Molecular phylogenetic analyses support (Ahyong & O'Meally 2004) or dispute (Chu et al. 2006; Tsang et al. 2008) family level status for the thaumastochelids. In the DNA tree of Ahyong & O'Meally (2004), which did not include *Acanthacaris*, *Thaumastochelopsis* is the sister taxon to the three nephropid genera analyzed. Our molecular analysis supports monophyly of thaumastochelids, similar to all previous morphological studies. However, it does not support family level status for thaumastochelids because they are nested within Nephropidae *sensu stricto*. The paraphyly of this taxon is also evident in the decapod tree based on nuclear protein coding genes (Tsang et al. 2008; Chu et al. this volume). We thus regard thaumastochelids as members of the Nephropidae. As with the putative Thaumastochelidae, the nephropid subfamilies Nephropinae (*Eunephrops*, *Homarus*, *Metanephrops*, *Nephrops*) and Thymopinae (*Nephropides*, *Nephropsis*, *Thymops*, *Thymopsis*) of Holthuis (1974) are not recovered by present results.

4.4 *Morphological convergence*

The present and recently published DNA studies facilitate detailed comparison with morphology-based phylogenies of nephropid genera. These agree in some aspects, for example, the placement of *Acanthacaris* (as discussed above) and *Eunephrops* and *Nephropides* forming a clade in some morphological studies (Tshudy & Babcock 1997; not Ahyong 2006) and in DNA studies (this study; Chu et al. 2006 [*Eunephrops* is a sister taxon to *Nephropides* + *Thymopides*]). However, morphological and DNA studies disagree in other aspects of nephropid phylogeny (discussed below), and these differences seem largely attributable to morphological convergence.

4.4.1 Homarus *and* Homarinus

A previous study based on 16S sequence data (Tam & Kornfield 1998; five nephropid genera) and also the present 12S–16S study position *Homarus* as the sister taxon to *Nephrops*, instead of *Homarinus*, as is common in morphological analyses (Tshudy & Babcock 1997; Ahyong 2006; Tshudy

et al. unpublished data). If these molecular results are interpreted to be more phylogenetically accurate than existing morphological studies (alpha-taxonomic and phylogenetic), then morphological similarities between *Homarus* and *Homarinus* are most parsimoniously explained as morphological convergence. *Homarus* and *Homarinus* are "plain-looking" nephropids that lack many of the distinguishing external features of other nephropid genera, features such as cephalothoracic carinae and spines, sculptured abdominal terga, and carinate claws. Ahyong (2006) found *Homarus* and *Homarinus* to be the most plesiomorphic of nephropids. Until recently, these two genera were considered congeneric. Kornfield et al. (1995) examined what were at that time three species of *Homarus* (*H. americanus*, *H. gammarus*, *H. capensis*) and removed *H. capensis* to a new genus, *Homarinus*, on the basis of DNA sequence comparisons and morphology. They reported 16S sequence (380 bp) divergence between *H. americanus* and *H. gammarus* at 1.3%, compared to average divergence between these and the "cape lobster" at 9.7% (Kornfield et al. 1995). Recent and present molecular analyses strongly support *Homarus* and *Homarinus* as having evolved in separate lineages, and both genera are "safely" nested in ornamented clades. Therefore, their morphologic similarities are interpreted as morphologic convergence.

4.4.2 Nephrops *and* Metanephrops

Similar to the *Homarus–Homarinus* example, *Nephrops* and *Metanephrops* are sister taxa in morphological analyses (Tshudy & Sorhannus 2000b; Tshudy et al., unpublished data) and are widely disparate in DNA-based trees (Chu et al. 2006; this study). In a morphological study parallel to this one (Tshudy et al., unpublished data), *Metanephrops* and *Nephrops* are the most derived nephropids and are sister taxa united by one unambiguous synapomorphy: the male pleopod 1 distal end is a posteriorly curving/terminating hook. There are several other obvious external similarities between these genera (ambiguous synapomorphies), which are apparently convergent. These similarities include their intermediate and lateral thoracic carinae, the complexly sculptured abdominal tergites, and their carinate and spiny claws. DNA analyses (Tam & Kornfield 1998 [16S]; present study [12S, 16S]) find *Nephrops* and *Metanephrops* well separated on the cladogram, indicating that the morphological similarities between these genera are the result of convergence.

5 CONCLUSIONS AND FUTURE WORK

This DNA analysis of clawed lobster genera facilitates detailed comparison with similarly comprehensive morphology-based topologies. There are major differences between the DNA and morphological results to date. *Eunephrops* and *Nephropides* form a sister group in some morphological studies and in DNA studies. Aside from that, there are conflicts at the level of family and genus.

Acanthacaris is determined to be the least derived of the genera in this analysis and is the sister group to all the nephropids, including the putative Thaumastochelidae. Published morphological studies have determined *Acanthacaris* to be more highly derived within the nephropids, and notably more so than the thaumastochelids.

Our molecular analysis supports monophyly of thaumastochelids, similar to all previous morphological studies. However, it does not support family level status for thaumastochelids, on the basis of their phylogenetic placement within Nephropidae. Thaumastochelidae should therefore be synonymized with Nephropidae.

Homarus and *Homarinus* form a clade in the morphological analyses, but our DNA analyses suggest they belong to different lineages, indicating that their similarities are the result of convergence. *Nephrops* and *Metanephrops*, likewise, form a clade in morphological analyses but are not closely related according to DNA analyses. Our molecular data suggest that *Homarus* and *Nephrops* are sister taxa, despite their being well separated in morphology-based trees.

Given the sensitivity of morphological analyses to taxon and character selection, which we interpret mainly to convergence, we should work toward further testing of DNA trees as guides to the phylogeny of extant and, ultimately, extinct lobsters. Thus far, sequences from four gene regions have been applied (12S, 16S, 18S, 28S), with as many as three in one analysis (Ahyong & O'Meally [2004] used 16S, 18S, and 28S). If the addition of new data (e.g., protein coding genes; see Tsang et al. 2008) stabilizes these trees, we could, through reverse extrapolation, infer which morphological characters are most phylogenetically reliable for analysis of extinct genera. Future work should also combine morphological and molecular data in a total evidence analysis.

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