548

Invertebrate Systematics, 2002, **16**, 839–847

Molecular phylogeny of the mud lobsters and mud shrimps (Crustacea : Decapoda : Thalassinidea) using nuclear 18S rDNA and mitochondrial 16S rDNA

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Abstract. Partial sequences of the 18S nuclear and 16S mitochondrial ribosomal genes were obtained for 14 species of thalassinidean shrimp (families Callianassidae, Laomediidae, Strahlaxiidae, Thalassinidae and Upogebiidae) and a further six species in related decapod infraorders (families Aeglidae, Astacidae, Lithodidae, Palinuridae, Raninidae and Scyllaridae). Maximum-likelihood and Bayesian analyses show equivocal support for the monophyly of the Thalassinidea, but show strong support for division of the infraorder into two major clades. This dichotomy separates representatives in the Upogebiidae, Laomediidae and Thalassinidae from those in the Strahlaxiidae and Callianassidae. The Laomediidae is shown to be paraphyletic, with the thalassinid species, *Thalassina squamifera*, being placed on a branch between *Axianassa* and a clade comprising *Jaxea* and *Laomedia*, the three current laomediid genera. For a monophyletic Laomediidae, the family Axianassidae should be resurrected for the genus *Axianassa*.

Introduction

The decapod infraorder Thalassinidea is a group of cryptic, marine, burrowing shrimp-like or lobster-like crustaceans that occur worldwide (with the exception of the coldest polar waters) in mostly shallow (<200 m) benthic habitats. Most species form complex burrow systems in soft sand or mud environments, but several taxa live in stony or coral-rubble areas and some even excavate burrows in living coral and sponge colonies (Dworschak 2000). There are currently 528 species in 84 genera spread across 11 recognised families of the three superfamilies: Axioidea, Thalassinoidea and Callianassoidea (Poore 1994; Dworschak 2000; P. Dworschak, personal communication).

Borradaile (1903: 551), in his seminal work on the classification of the Thalassinidea, presented a 'genealogical tree' of proposed relationships among 12 known genera in the four families recognised at that time. His tree (reproduced in Fig. 1*A* with current family designations) separates these genera into five major clades corresponding with the families Axiidae (*Axiopsis*, *Axius*, *Calocaris*, *Scytoleptus*), Laomediidae (*Jaxea*, *Laomedia*), Thalassinidae (*Thalassina*) and the two subfamilies, Callianassinae (*Callianassa*, *Callianidea*, *Glypturus*) and Upogebiinae (*Gebicula*, *Upogebia*), of the Callianassidae. Later, Gurney (1938: 343) used larval morphology to present an intuitive tree of relationships among four thalassinidean families and the Anomura (Fig. 1*B*). A period of 56 years elapsed before any further publications on the phylogenetic relationships within this obscure, but taxonomically large, infraorder emerged. Poore (1994: 120) published a comprehensive revision of the members of the Thalassinidea based on morphology, including a new classification scheme, keys to families and genera and a phylogenetic tree (reproduced in Fig. 1*C* to the family level only). This tree was the first cladistic analysis of the infraorder as a whole and separated the 22 selected genera into the currently recognised 11 families and three superfamilies (despite showing a basal dichotomy with only two major clades). He also proposed a monophyletic origin for the infraorder, with the Anomura being the closest sister-group.

Several papers have been published employing cladistic analyses of relationships among or within genera of certain thalassinidean families. These investigations cover the families Axiidae and Calocaridiidae (Kensley 1989), Callianideidae (Kensley and Heard 1991), Callianassidae (Staton and Felder 1995; Staton *et al.* 2000; Tudge *et al.* 2000) and Ctenochelidae (Tudge *et al.* 2000).

In some cases, thalassinidean representatives have been used as either ingroup or outgroup taxa for larger cladistic analyses of various Decapoda; these include studies using morphological characters by Martin and Abele (1986), Scholtz and Richter (1995) and Tudge (1997). Several

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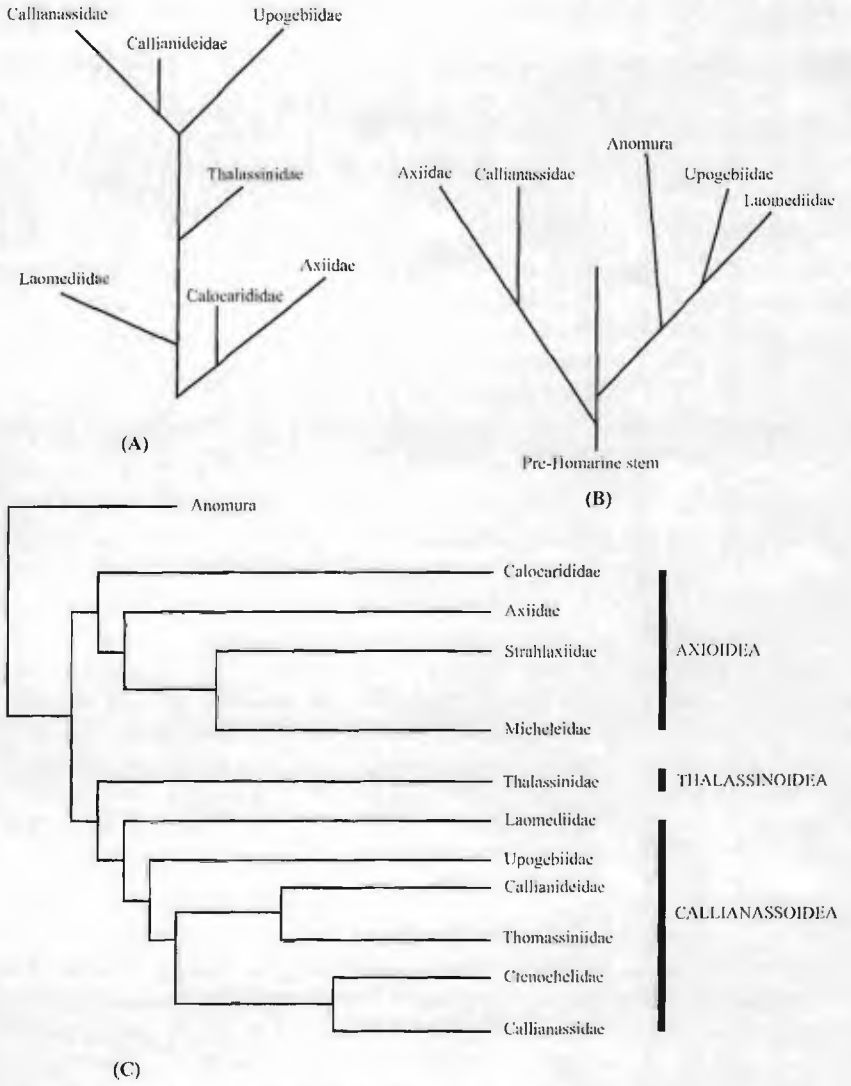

Fig. 1. (*A*) Genealogical relations between families in the Thalassinidea according to Borradaile (modified from Borradaile 1903). (*B*) Intuitive tree of relationships between some thalassinideans based on larval characters (modified from Gurney 1938). (*C*) Cladogram of phylogenetic relationships between 11 families of the Thalassinidea based on morphological characters (modified from Poore 1994). Note: the trees of both Borradaile and Poore have been modified to show taxonomic relationships at the family-level or higher.

molecular phylogenetic analyses of various decapod taxonomic groups have also included thalassinidean taxa for comparison with anomurans (Spears and Abele 1988; Pérez-Losada *et al.* 2002), astacideans (Crandall *et al.* 2000) and crab-like decapods (Morrison *et al.* 2002). Also, see the synopsis of recent research into decapod phylogenetics by Schram (2001) for a review of the different approaches and data sets being applied to the field.

The contribution of the small-subunit 18S ribosomal (r)DNA nuclear gene to crustacean phylogeny is well known and has been useful in investigating relationships across a wide variety of groups (Spears and Abele 1988, 1997, 1998; Abele *et al.* 1989, 1992; Kim and Abele 1990; Abele 1991; Spears *et al.* 1992; Crandall *et al.* 2000; Morrison *et al.* 2002; Pérez-Losada *et al.* 2002). Similarly, the mitochondrial 16S rDNA gene has been regularly used to investigate decapod relationships (Cunningham *et al.* 1992; Crandall and Fitzpatrick 1996; Tam *et al.* 1996; Ovenden *et al.* 1997; Kitaura *et al.* 1998; Tam and Kornfield 1998; Crandall *et al.* 1999, 2000; Schubart *et al.* 2000, 2001; Duffy *et al.* 2000; Morrison *et al.* 2002). Different regions of ribosomal genes evolve at varying rates, making these useful molecular markers across a broad taxonomic spectrum.

This paper presents the results of a phylogenetic analysis of partial sequences of mitochondrial 16S rDNA and nuclear 18S rDNA from 14 thalassinideans in five families

(Callianassidae, Laomediidae, Strahlaxiidae, Thalassinidae and Upogebiidae). Six other decapod taxa from the Astacidea, Palinura, Brachyura and Anomura are included in the analysis as outgroups. Similarities and differences to previous evolutionary trees of the Thalassinidea, most notably those of Borradaile (1903), Gurney (1938), Poore (1994) (Figs 1*A–C*) and Tudge *et al.* (2000), are discussed.

Materials and methods

Specimen collection

With the exception of the *Raninoides* specimen from 1991, animals for the genetic analysis were mostly collected over a 5-year period (1995–1999) from a variety of subtidal and intertidal locations worldwide (Table 1), but with an emphasis on coastal Australia and the United States. The intertidal specimens were collected by 'yabby' pump, digging or by hand and the subtidal specimens were trawled or dredged.

Table 1. Specimens, collection, voucher and GenBank-sequence information

Higher-level classification from Martin and Davis (2001). Abbreviations: AMNH, American Museum of Natural History (New York); MV, Museum Victoria; Qld, Queensland; USA, United States of America; USNM, National Museum of Natural History (Washington DC); Vic, Victoria.

Astacidea
- Cambaridae
  - *Procambarus clarkii* (Girard, 1852). Louisiana, USA, Jan. 1998. Voucher: AMNH 17874. GenBank sequences: AF436040 (16S), AF436001 (18S)

Palinura
- Palinuridae
  - *Panulirus argus* (Latreille, 1804). GenBank sequences: AF337966 (16S), U19182 (18S)
- Scyllaridae
  - *Thenus orientalis* (Lund, 1793). Gulf of Carpentaria, Qld, Australia, 1997. Voucher: not available

Brachyura
- Raninidae
  - *Raninoides louisianensis* Rathbun, 1933. Gulf of Mexico, USA, 1991. Voucher: T. Spears personal collection. GenBank sequences: AF436044 (16S), AF436005 (18S)

Anomura
- Aeglidae
  - *Aegla uruguayana* Schmitt, 1942. San Antonio de Areco, Buenos Aires, Argentina, 10 Mar. 1996. Voucher: C. Cunningham personal collection. GenBank sequences: AF436051 (16S), AF436012 (18S).
- Lithodidae
  - *Cryptolithodes typicus* Brandt, 1849. British Columbia, Canada. Voucher: S. Zaklan personal collection. GenBank sequences: AF425325 (16S), AF436019 (18S)

Thalassinidea

Superfamily Thalassinoidea
- Thalassinidae
  - *Thalassina squamifera* de Man, 1915. Town of 1770, Qld, Australia, 29 Dec. 1997. Voucher: MV J41662

Superfamily Callianassoidea
- Callianassidae
  - *Biffarius arenosus* (Poore, 1975). Dunwich, Qld, Australia, 21 May 1997. Voucher: MV J40669
  - *Biffarius delicatulus* Rodrigues & Manning, 1992. Fort Pierce, Florida, USA, 9 Jun. 1999. Voucher: USNM 309754
  - *Callianassa filholi* A. Milne Edwards, 1878. Otago Harbour, New Zealand, 14 Nov. 1997. Voucher: MV J44818
  - *Callichirus major* (Say, 1818). Isles Dernieres, Louisiana, USA, 27 Mar. 1996. Voucher: MV J39044. GenBank sequences: AF436041 (16S), AF436002 (18S)
  - *Neocallichirus rathbunae* (Schmitt, 1935). Fort Pierce, Florida, USA, 8 Jun. 1999. Voucher: USNM 309751
  - *Neotrypaea californiensis* (Dana, 1854). Marina del Rey, California, USA, 20 Jun. 1997. Voucher: A. Harvey personal collection. GenBank sequences: AF436042 (16S), AF436003 (18S)
  - *Sergio mericeae* Manning & Felder, 1995. Fort Pierce, Florida, USA, 8 Jun. 1999. Voucher: USNM 309755
- Laomediidae
  - *Axianassa australis* Rodrigues & Shimizu, 1992. São Sebastião, São Paulo, Brazil, 20 Jul. 1997. Voucher: MV J44613
  - *Jaxea nocturna* Nardo, 1847. Loch Sween, Argyll, Scotland, 10 Nov. 1996. Voucher: MV J39045. GenBank sequences: AF436046 (16S), AF436006 (18S)
  - *Laomedia healyi* Yaldwyn & Wear, 1970. Western Port Bay, Vic., Australia, 16 Apr. 1997. Voucher: MV J40697
- Upogebiidae
  - *Gebiacantha plantae* (Sakai, 1982). Yorke Island, Qld, Australia, 26 Feb. 1998. Voucher: MV J44914
  - *Upogebia affinis* (Say, 1818). Fort Pierce, Florida, USA, 15 Nov. 1995. Voucher: MV J40668. GenBank sequences: AF436047 (16S), AF436007 (18S)

Superfamily Axioidea
- Strahlaxiidae
  - *Neaxius glyptocercus* von Martens, 1868. Dunwich, Qld., Australia, 21 May 1997. Voucher: MV J39643

Molecular protocols

Tissue samples were preserved in 95% ethanol. DNA was isolated from fresh, frozen or ethanol-preserved specimens by grinding small fragments of muscle tissue in a buffer (0.1 M EDTA, 0.01 M Tris pH 7.5, 1% SDS, Palumbi *et al.* 1991), followed by phenol-chloroform-isoamyl alcohol extraction and precipitation with 7.5 M ammonium acetate and cold isopropanol as described by Palumbi *et al.* (1991). Partial sequences of mitochondrial 16S rDNA were amplified using rDNA primers (LR-N-13398, alias 16Sar, and LR-J-12887, alias 16 Sbr, Simon *et al.* 1994). Most of the nuclear 18S rDNA gene was amplified using 18E-F (5′-CTGGTTGATCCTGCCAGT-3′) and 18SR3 (5′-TAATGATCCTTCCGCAGGTT-3′). The amplification and sequencing of the two genes (16S and 18S) was identical (except where indicated) and entailed the following regime. Polymerase chain reaction (PCR) was carried out on a Perkin Elmer GeneAmp PCR System 9600 (www.pecorporation.com) using 50 µL reactions consisting of 1 µL template DNA, 3 µL primers, 4.5 µL 10× reaction buffer, 5 µL Taq polymerase, 4 µL deoxynucleoside triphosphates, 5 µL $MgCl_2$ and 24.5 µL water. Amplification involved 20 sec denaturation at 94°C, 1.5 min annealing at 50°C (40°C for 16S) and 2.5 min of extension at 72°C for 35 cycles. The PCR product (3 µL) was visualised, via electrophoresis, on a 1% agarose gel using ethidium bromide staining. The remaining product (45 µL) was purified using the Wizard® PCR Preps (www.promega.com) DNA Purification System. Applied Biosystems, Inc. (ABI) (www.appliedbiosystems.com) Prism BigDye Terminator Cycle Sequencing Ready Kits and an ABI Prism 3700 DNA Analyzer were used for sequencing. Sequences are available from the authors (CWC).

Sequence alignment and phylogenetic analysis

Sequences were aligned using Clustal X (Thompson *et al.* 1994, 1997) with gap insertion and extension costs at 10 and 5, respectively, and regions of uncertain homology removed before phylogenetic analysis. Maximum-likelihood (ML) analyses using heuristic searches and TBR branch swapping were applied to the aligned sequences using PAUP* 4.0 (Swofford 2001). For both maximum-likelihood and Bayesian analyses, we used the general-time-reversible (GTR) model to estimate the proportion of invariant sites and the alpha parameter of the gamma distribution from the data (the best-fit model using ModelTest, Posada and Crandall 1998). Incongruence testing was carried out under the parsimony criterion using the incongruence length difference (ILD) test (Farris *et al.* 1995), as implemented in PAUP* 4.0.

The Bayesian analyses were performed using the program MRBAYES 1.11 (Huelsenbeck and Ronquist 2001). MRBAYES uses a metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm to sample from the posterior distribution. Each Markov chain was started from a random tree and run for 5 million cycles. The chain was sampled every 500 cycles in order to minimise the size of the output files and to ensure that these samples were independent. Four chains were run simultaneously with a 'temperature' of 0.2. The initial 40% of cycles were discarded as burn-in and convergence was checked by ensuring that the likelihood values of sampled trees had approached a level distribution. Each analysis was performed twice to ensure that convergence was repeatable.

Tests of alternative topologies were performed using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999), which does not require that the hypotheses be designated *a priori*. Probability values were calculated in PAUP* 4.0 using 1000 RELL replicates to obtain a distribution.

Results

After removing regions of uncertain alignment, our aligned sequences consisted of 1731 base pairs (bp) of nuclear 18S rDNA and 327 bp of mitochondrial 16S rDNA for a total of 2058 bp. Our final alignments are available from the authors (CWC). An ILD test (Farris *et al.* 1995) showed no significant incongruence ($P > 0.05$) and the two genes were analysed separately and together.

For both genes, the best-fit model found using ModelTest under maximum-likelihood was GTR + gamma + inv. Bootstrap consensus trees for both genes, analysed separately for the maximum-likelihood bootstrap and Bayesian analyses, are presented in Fig. 2. There is weak support in the 18S analysis for a monophyletic Thalassinidea (70% Bayes, 42% bootstrap). Although Bayesian analysis of the 16S gene showed rather strong support for a paraphyletic Thalassinidea (85%), there is little ML bootstrap support for a paraphyletic Thalassinidea (33%). The conflict between the 18S and 16S genes is not strong (also reflected by a non-significant ILD test of $P > 0.05$) and they were combined for further analysis.

The ML analysis found a tree (Fig. 3) with Ln likelihood = –8184.94 that weakly supports a paraphyletic Thalassinidea (76% Bayes, 56% bootstrap). A monophyletic Thalassinidea could not be rejected by an SH test.

Discussion

In these analyses, monophyly of the Thalassinidea was equivocal, being weakly supported by the 18S dataset only (Fig. 2, left side) and not by the 16S dataset (Fig. 2, right side) or the combined analysis (Fig. 3). Poore (1994; Fig. 1*C*) found a monophyletic Thalassinidea based on two morphological synapomorphies (reduction of pleurobranchs on gills to seven or less and presence of a setose lower margin on the propodus and carpus of pereopod 2), but a molecular study by Morrison *et al.* (2002) found strong support for non-monophyly of the Thalassinidea (89% ML bootstrap support). The latter study included more sequences (16S and 18S, as well as partial sequences of mitochondrial COII and nuclear 28S ribosomal DNA), but fewer thalassinidean taxa than the present study. It is possible that the more rapidly evolving sequences used in the Morrison *et al.* (2002) study give a more reliable indication of relationships than just the two genes used in the present study.

Between the Bayesian and the ML analyses there is no clear candidate for a sister-group to the Thalassinidea as a whole (Figs 2, 3). Interestingly though, the Bayesian analysis found strong (90%) support for a monophyletic sister-group to some taxa in the Thalassinidea, including the spiny and slipper lobsters (*Panulirus* and *Thenus*), the anomurans (*Aegla* and *Cryptolithodes*) and the brachyuran (*Raninoides*). Although this is intriguing, the weak support for this clade in bootstrapping (59%) suggests that further data may be necessary to test this hypothesis.

Within the Thalassinidea, we found two strongly supported major clades (Figs 2, 3). The first clade includes a monophyletic Callianassidae as the sister-group to a representative in the axioid family, Strahlaxiidae (*Neaxius glyptocercus*). Within the subfamily Callianassinae, the genus

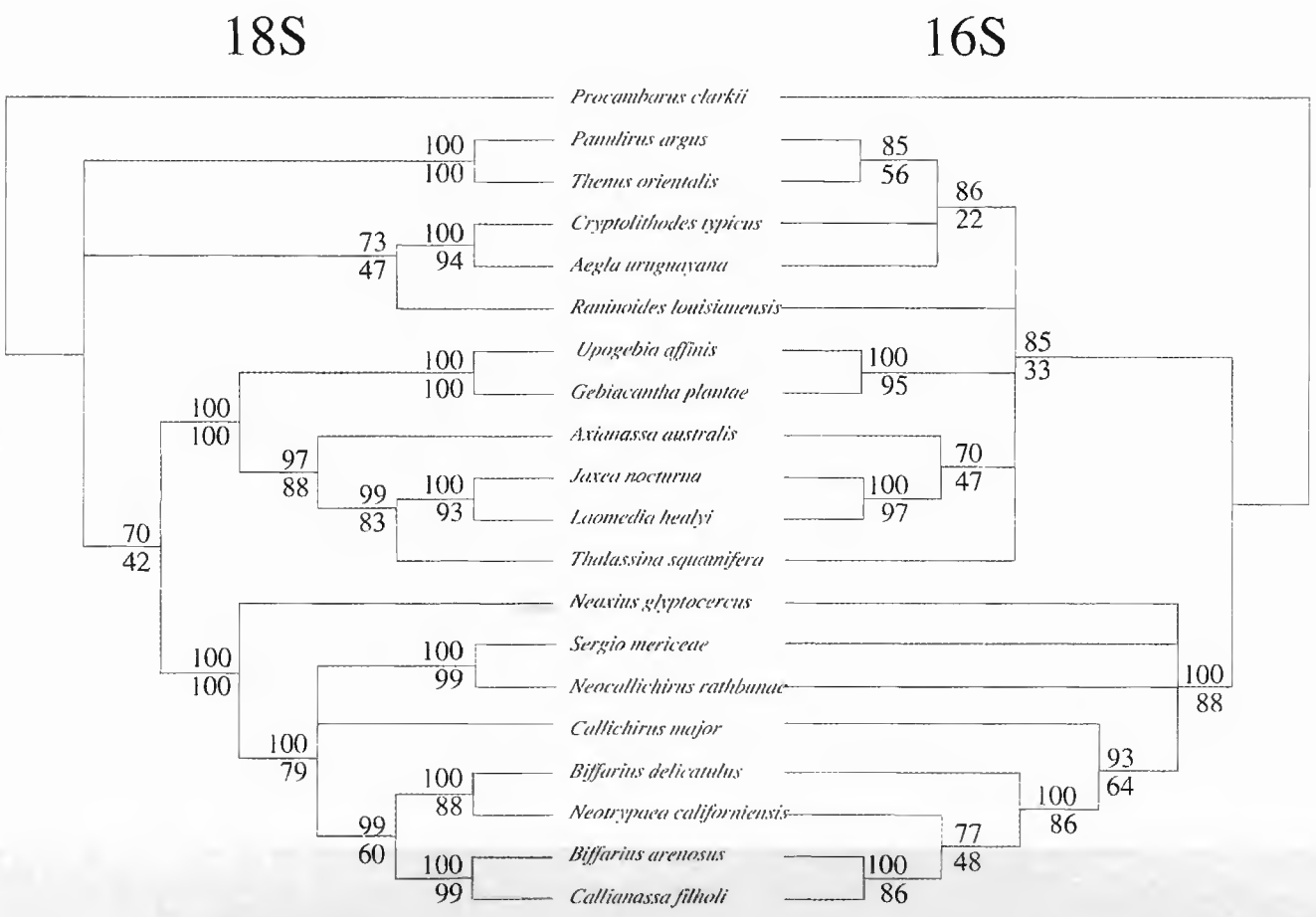

Fig. 2. A combined figure showing the opposed consensus trees for both Bayesian and maximum-likelihood analyses of the 18S data set (left side) and the 16S data set (right side). Percentage support for Bayesian posterior probabilities (upper) and maximum-likelihood bootstrap values (lower) are given for each node. Best-fit model = GTR + gamma + inv.

Biffarius is paraphyletic, with representatives of the genus apparently grouping according to biogeography, uniting the American taxa *Biffarius delicatulus* and *Neotrypaea californiensis*, and uniting the antipodean taxa, *Biffarius arenosus* and *Callianassa filholi*. These associations, although at first appearing enigmatic, should be viewed in light of comments by Tudge *et al.* (2000) that generic relationships within the family Callianassidae should be treated with some caution pending a re-diagnosis of the inclusive taxa, the subfamily Callianassinae and the nebulous genus *Callianassa* in particular. The apparent biogeographic associations between the four species mentioned above are contradictory to those proposed for the same taxa in the morphological analysis (Tudge *et al.* 2000), where the two *Biffarius* species are included in a monophyletic clade (only 59% supported) and *C. filholi* and *N. californiensis* are in another smaller monophyletic clade (100% supported). The same morphological analysis by Tudge *et al.* (2000) grouped these four callianassine taxa into a monophyletic clade and indicated a comparable lack of resolution between this clade and the callichirine taxa, *Callichirus*, *Sergio* and *Neocallichirus*. The monophyletic Callianassidae in this molecular analysis (Figs 2, 3) is supported by at least 10 morphological characters (see family diagnosis in Poore 1994). In addition to Tudge *et al.* (2000), a recent synopsis of the family Callianassidae has been provided by Sakai (1999). His paper indicates a polyphyletic family, extensively synonymises members of the Callianassoidea and has been considered of limited value in helping to elucidate the relationships in this group of thalassinideans (Tudge *et al.* 2000; Poore 2000).

The large clade constituted by the Callianassidae and Strahlaxiidae in this molecular analysis (Figs 2, 3) is also supported by six morphological synapomorphies. Three of these synapomorphies can be found in the analysis of Poore (1994), but are also shared by representatives in several other thalassinoid families (Callianideidae, Ctenochelidae, Micheleidae and Thomassiniidae) for which tissue was not available for this molecular analysis. These characters are (*1*) the presence of dense tufts of setae on abdominal somites 3–5 (*2*) the absence of the appendix interna on male pleopod 1 and (*3*) a similar absence on male pleopod 2. Three additional morphological synapomorphies were found to support the

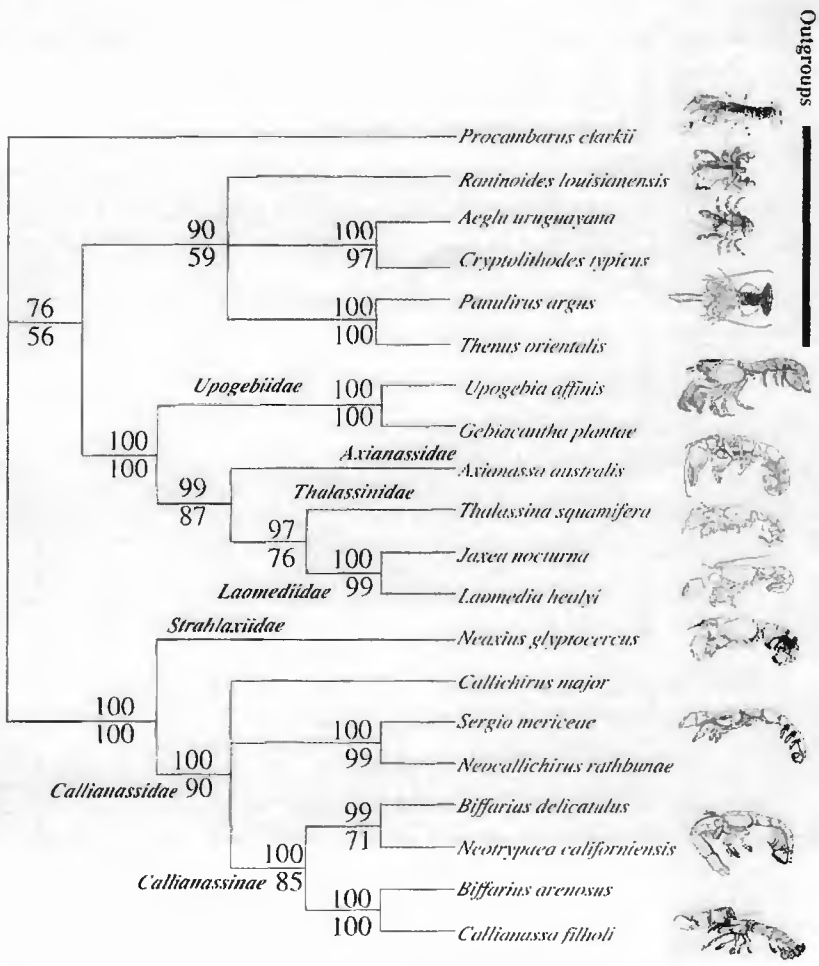

Fig. 3. A composite of the consensus trees obtained from the Bayesian analysis and the single maximum-likelihood tree for the combined 16S and 18S data sets. Percentage support for Bayesian posterior probabilities (upper) and maximum-likelihood bootstrap values (lower) are given for each node. Best-fit model = GTR + gamma + inv. Some higher taxonomic categories are provided on specific clades and the six outgroup taxa are indicated. Decapod images are modified from (top to bottom): Hobbs 1981; Rathbun 1937; Jara and Palacios 1999; Bliss 1982; Holthuis 1991; Rodrigues and Shimizu 1992; Sakai 1992; Yaldwyn and Wear 1970; Kensley *et al.* 2000; Felder and Felgenhauer 1993; Hart 1982; Holthuis 1991. Note: images are not necessarily the same species as used in the analysis, but are representative of the genus, or a closely related genus.

association of the Callianassidae with the Strahlaxiidae in an unpublished analysis by the senior author, which did not use the exact corresponding taxa. These characters are (*4*) a chelate pereopod 2 with the dactylus as long as the fixed finger (*5*) the absence of spiniform setae on the dactylus of pereopods 3 and 4 and (*6*) a row of lateral plumose setae on abdominal somite 2.

The second major clade in Fig. 2 (left side only) and Fig. 3 includes three genera currently in the Laomediidae (*Jaxea*, *Laomedia*, *Axianassa*), as well as representatives of the Thalassinidae and Upogebiidae. Within this clade, the placement of the thalassinid, *Thalassina squamifera*, makes the Laomediidae paraphyletic, or monophyletic with the inclusion of *Thalassina*. This latter, alternative hypothesis, although lacking any convincing morphological support (see below), could not be rejected by an SH test of the molecular data. The paraphyly of the laomediids, suggested in the present analysis, resurrects an interesting issue on the validity of the monotypic thalassinidean family, Axianassidae Schmitt, 1924. Kensley and Heard (1990) succinctly summarised the history of the debate on whether the Axianassidae is a valid family in the Thalassinidea, and listed the supporters of the Axianassidae (Gurney 1938; Wear and Yaldwyn 1966; Goy and Provenzano 1979; Poore and Griffin 1979) and those who believe the genus *Axianassa* is one of five in the Laomediidae (de Man 1928;

Balss 1957; Le Loeuff and Intes 1974; Ngoc-Ho 1981). Our data support retention of the family Axianassidae for the genus *Axianassa*, with *Jaxea* and *Laomedia* remaining in the Laomediidae. As well as the molecular evidence for a valid Axianassidae presented here (Figs 2, 3), we have identified 10 morphological characters (six of them apomorphies) in which *Axianassa* differs from *Jaxea* and *Laomedia*. These apomorphic characters are: (*1*) the linea thalassinica displaced dorsally (possible autapomorphy); (*2*) absence of lateral lobes on abdominal somite 1; (*3*) linear epipods on the gills; (*4*) reduction (or absence) of an exopod on maxilliped 3; (*5*) unequal chelae on pereopod 1; and (*6*) dense tufts of lateral setae on abdominal somites 3–5.

The association of the Thalassinidae and the Laomediidae (the 76% ML bootstrap supported node with *Thalassina*, *Jaxea*, and *Laomedia* in Fig. 3) is interesting, but lacks strong morphological support. In fact, only two synapomorphies(?), the posterior margin of the carapace with strong lateral lobes and both anterior and posterior teeth on the mandibular incisor, can be gleaned from the cladistic analysis by Poore (1994). The former is shared with representatives of the Axioidea, whereas the latter character is shared with members of the Thomassiniidae (once again taxa missing from the current molecular analysis). Some support, however, for this laomediid–thalassinid association, is provided by studies of larval characters (Sankolli and Shenoy 1979) and gill-cleaning structures and mechanisms (Batang and Suzuki 1999; Batang *et al.* 2001). Although the latter authors found gill-cleaning characters to be conservative at the family level, there is still debate over their utility in phylogenetic studies (Poore 1994; Suzuki and McLay 1998).

No unique morphological synapomorphies support the major monophyletic clade containing the Upogebiidae, Axianassidae, Thalassinidae and Laomediidae, seen in Figs 2, 3, even though there is strong molecular support in both Bayesian and ML analyses. In the morphological analysis of Poore (1994; Fig. 1*C*), *Upogebia*, *Thalassina* and *Laomedia* do not form a distinct clade, but three morphological characters variously ally these three taxa, which are part of a monophyletic clade (with other taxa) in the current molecular analysis. These are (*1*) the rostrum augmented with ridges (with the exception of the Laomediidae), (*2*) a cylindrical carpus and propodus on pereopod 1 (a symplesiomorphy shared with some axioids and some outgroup representatives) and (*3*) the loss or reduction of the male first pleopod (except in Thalassinidae). This last morphological character is a possible synapomorphy shared with some callianassids (Poore 1994) or a symplesiomorphy also shared with some members of the outgroup.

In summary, the monophyly of the Thalassinidea suggested from a cladistic analysis of morphological characters (Poore 1994; Fig. 1*C*) is only weakly supported by the 18S gene sequence data (Fig. 2, left side) and unsupported by the 16S data (Fig. 2, right side) and the combined analysis of both genes (Fig. 3). Borradaile (1903), in his paper on thalassinidean classification, did not implicitly state that the group is monophyletic, but inferred this in his intuitive genealogical tree (Fig. 1*A*) and Gurney (1938; Fig. 1*B*) advocates paraphyly for the Thalassinidea he studied. Although a paraphyletic Thalassinidea has some support in the present molecular analysis, the question of monophyly for this enigmatic group will require more analysis of both morphological and molecular data sets to resolve.

A dichotomy within the Thalassinidea was previously suggested by Poore (1994) based on morphological data, but neither of his major clades corresponds to ours. In contrast, the dichotomy described by Gurney (1938; Fig. 1*B*) based on larval characteristics is reminiscent of the relationships seen in our 16S tree and combined gene tree (Figs 2 (right side), 3). This closer relationship of the thalassinidean families Laomediidae and Upogebiidae with the Anomura in Gurney's proposed classification (Fig. 1*B*) is only weakly supported (under both Bayesian and ML analyses) here and the anomuran representatives are always part of a larger decapod sister-clade (Figs 2, 3). Since our analysis only includes half of the families in the Thalassinidea, further sampling will be necessary to determine the composition of these major clades, which appear with regularity in cladistic analyses of this group.

The three monophyletic superfamilies (Axioidea, Thalassinoidea and Callianassoidea) indicated in the classification of Poore (1994) are not maintained as monophyletic entities in our analysis. In fact, in Poore's phylogeny (Fig. 1*C*), these superfamilies are not adequately represented by monophyletic clades at the same level either. This discrepancy was noted by Martin and Davis (2001), who followed Poore's revision in their recent work. Until better taxonomic congruence is achieved, and more of the 11 families are represented in molecular analyses such as the present study, direct comparisons will remain challenging.

Acknowledgments

This project was initiated when the senior author (CCT) was an Australian Research Council Postdoctoral Research Fellow (1996–1999) at Museum Victoria, Melbourne, Australia, and the authors acknowledge the assistance of Gary Poore, Les Christidis, Janette Norman and Megan Osborne from this institution for project support, access to laboratory facilities (CCT) and training in molecular techniques (CCT). The authors thank Cheryl Morrison, Bo Kong and Todd Oakley for technical support within the Department of Zoology at Duke University. Also, Kristian Fauchald and Rafael Lemaitre (Department of Systematic Biology, National Museum of Natural History, Smithsonian Institution) are thanked for supporting the Research

Associate status of CCT during the completion of the project. The following colleagues are thanked for collecting, assistance in collecting, or supplying specimens for this study: Katrin Berkenbush, David Brewer, Vania Coelho, Darryl Felder, Alan Harvey, Woody Lee, Rafael Lemaitre, Cheryl Morrison, Louise Nickel, Philip O'Neil, Gary Poore, John Salini, Jo Taylor and Stef Zaklan. The improvement of the manuscript by three anonymous reviewers is gratefully acknowledged. This is contribution no. 548 of the Smithsonian Marine Station at Fort Pierce, Florida. The project was funded by NSF DEB–9615461 (to CWC).

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Manuscript received 30 April 2002; revised and accepted 15 October 2002.