Evolution and phylogeny of the mud shrimps (Crustacea: Decapoda) revealed from complete mitochondrial genomes

Feng-Jiau Lin1†, Yuan Liu2†, Zhongli Sha2, Ling Ming Tsang3, Ka Hou Chu2, Tin-Yam Chan4, Ruiyu Liu2 and Zhaoxia Cui2*

Abstract

Background: The evolutionary history and relationships of the mud shrimps (Crustacea: Decapoda: Gebiidea and Axiidea) are contentious, with previous attempts revealing mixed results. The mud shrimps were once classified in the infraorder Thalassinidea. Recent molecular phylogenetic analyses, however, suggest separation of the group into two individual infraorders, Gebiidea and Axiidea. Mitochondrial (mt) genome sequence and structure can be especially powerful in resolving higher systematic relationships that may offer new insights into the phylogeny of the mud shrimps and the other decapod infraorders, and test the hypothesis of dividing the mud shrimps into two infraorders.

Results: We present the complete mitochondrial genome sequences of five mud shrimps, *Austinogebia edulis*, *Upogebia major*, *Thalassina kelanang* (Gebiidea), *Nihonotrypaea thermophilus* and *Neaxius glyptocercus* (Axiidea). All five genomes encode a standard set of 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes and a putative control region. Except for *T. kelanang*, mud shrimp mitochondrial genomes exhibited rearrangements and novel patterns compared to the pancrustacean ground pattern. Each of the two Gebiidea species (*A. edulis* and *U. major*) and two Axiidea species (*N. glyptocercus* and *N. thermophilus*) share unique gene order specific to their infraorders and analyses further suggest these two derived gene orders have evolved independently. Phylogenetic analyses based on the concatenated nucleotide and amino acid sequences of 13 protein-coding genes indicate the possible polyphyly of mud shrimps, supporting the division of the group into two infraorders. However, the infraordinal relationships among the Gebiidea and Axiidea, and other reptants are poorly resolved. The inclusion of mt genome from more taxa, in particular the reptant infraorders Polychelida and Glypheidea is required in further analysis.

Conclusions: Phylogenetic analyses on the mt genome sequences and the distinct gene orders provide further evidences for the divergence between the two mud shrimp infraorders, Gebiidea and Axiidea, corroborating previous molecular phylogeny and justifying their infraordinal status. Mitochondrial genome sequences appear to be promising markers for resolving phylogenetic issues concerning decapod crustaceans that warrant further investigations and our present study has also provided further information concerning the mt genome evolution of the Decapoda.

Keywords: Mud shrimps, Mitochondrial genome, Gene order, Evolution, Phylogenetics

* Correspondence: zhxcui@qdio.ac.cn
†Equal contributors
2EMBL, Institute of Oceanology, Chinese Academy of Sciences, Qingdao
266071, China
Full list of author information is available at the end of the article

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Background

Decapoda is one of the most diverse groups of crustaceans, with over 15,000 extant species in 180 families [1]. Seven main groups (with the ranks of sub- or infra-order) are generally recognized in Decapoda. They are Dendrobranchiata (e.g. penaeoid shrimps and allies), Caridea (caridean shrimps), Stenopodidea (stenopodid shrimps), lobsters (Macrura Reptantia), mud shrimps or ghost shrimps (Thalassinidea or Gebiidea + Axiidea), Anomura (hermit crabs and allies) and Brachyura (true crabs). The phylogenetic relationships amongst these groups within the Decapoda and even the monophyletic status of these groups have long been debated amongst carcinologists and general consensus has yet to be reached, with recent morphological cladistic and molecular analyses still showing contrasting results (Figure 1). One of the most controversial recent findings is that the mud shrimps are not monophyletic [1-8], with some of them representing the sister taxon of the other Reptantia (= non-shrimp like decapod crustaceans).

Mud shrimps have a worldwide distribution from shallow to deep waters, and more than 600 extant species have been described to date [1]. The classification scheme of mud shrimps have been in flux at all levels. They are often considered to be a monophyletic group up to the rank of infraorder, i.e., Thalassinidea [9-16]. According to different authors, these animals have been treated under Anomura [17-21], as an independent group within the Reptantia [10,11,14,22,23], or aligned with the lobsters [24]. While some authors [25-29] had long questioned the monophyly of Thalassinidea and divided it into two groups (namely Gebiidea and Axiidea), the monophyly of Thalassinidea has been supported by some morphological cladistic analyses [9,10,12,14,16], molecular data [11,30] or combined morphological and molecular analysis [15]. Nevertheless, the latest molecular analyses [3-8] mostly concur in the separation of Thalassinidea.

In most molecular analyses, partial DNA sequences are used to resolve the phylogenetic relationships of decapod crustaceans [3,11,15,22-34], but they are often too short to contain a sufficient amount of genetic variation for resolving higher systematics [5,35]. In the previous studies involving mud shrimps [3,4,6,7,11,15,30], the total length of partial sequences used is less than 5300 bp. The animal mitochondrial (mt) DNA is a small, extrachromosomal, and circular double-stranded DNA molecule of 12–20 kb in size, and usually contains the same set of 37 genes, including 13 protein-coding genes, two ribosomal RNA genes and 22 transfer RNA genes [36-38]. Recent advances in DNA sequencing technology have allowed rapid, cost-effective sequencing of the complete mtDNA genome. And it has become increasingly popular in studies of molecular evolution, phylogeography, and phylogenetic relationships.

Figure 1 Hypotheses of higher-level decapod relationships based on recent morphology analyses (A-C), molecular data (D-J), combined morphology and molecular data (K) and latest complete mtDNA sequence (L). Taxa name following De Grave et al. [1] instead of original usages. *Thalassinidea refers to treat members of Gebiidea and Axiidea as forming a monophyletic group. Traditionally, lobsters (Macrura Reptantia) refer to members of Astacidea, Achelata, Glypheidea and Polychelida, while Procarididea is included in Caridea.
at various taxonomic levels [38-43], mainly because of its maternal inheritance, the presence of strictly orthologous genes evolving at different rates, and lack of genetic recombination [38,44-46]. Complete mtDNA sequences provide sets of genome-level characteristics, such as gene rearrangement, which is rather conserved within some major metazoan lineages, and therefore can be especially powerful in resolving systematic relationships among higher taxa [3,40,42,47-49].

Complete mitochondrial genome sequences are now available for 37 decapod crustaceans (April, 2012; http://www.ncbi.nlm.nih.gov, with a sergesteid species Actetes chinensis with mitogenome reported [50] but not yet available from GenBank) that represent all the main groups. However, the latest phylogenetic reconstruction of decapod crustaceans using complete mitochondrial genome sequences [51,52] still has low resolution in most of the deep branches, notably with the status of Stenopodidea, lobsters and mud shrimps unresolved. Moreover, only a single species of mud shrimp collected from Korea, namely Upogebia major (De Haan, 1841) belonged to Gebiidea, has been sequenced for mitochondrial genome [53].

In this paper, we report the complete mitochondrial genomes of five thalassinidean species with representatives from both Gebiidea and Axiidea. They are Austinogebia edulis (Ngoc-Ho and Chan 1992), Upogebia major (from China) and Thalassina kelanang Moh and Chong, 2009 of Gebiidea, and Nihonotrypaea thermophilus Lin, Komai and Chan, 2007 and Neaxius glyptocercus (Von Martens, 1868) of Axiidea. Considering the difference in sampling location and sequence variation, we only included the mitochondrial genome of Upogebia major we sequenced in the analysis. The mitochondrial genome structure of these five mud shrimps were compared with those of other decapods. The gene rearrangement occurred in mud shrimps were identified. Moreover, the infraorder status of Axiidea and Gebiidea was analyzed based on all 50 malacostracan mitochondrial genomes currently available.

**Results**

**Genome composition**

The complete mtDNA sequences of A. edulis, U. major, T. kelanang, N. glyptocercus and N. thermophilus were determined to be 15,761, 16,143, 15,528, 14,909 and 15,240 bp long, respectively (Additional file 1). They all contained 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNA), 22 transfer RNA genes (tRNA) and a putative control region as in other metazoans (Figure 2; Additional files 2, 3, 4, 5, 6). The structural organizations of the five mitochondrial genomes are shown in Figure 2.

The overall A + T content of A. edulis mtDNA is 73.6%, higher than that of other decapod species except Scylla tranquebarica (73.8%) (see Additional file 1). The overall A + T content of U. major, T. kelanang, N. glyptocercus and N. thermophilus ranged from 66.3-70.7%, similar to other decapods (see Additional file 1). This pattern of base composition in five mud shrimps held for the protein-coding, rRNA, tRNA genes, and the control region when considered separately.

For the 13 PCGs of five mitochondrial genomes, nine protein-coding genes (atp6, atp8, cox1, cox2, cox3, cob, nad2, nad3, and nad6) were encoded on the H-strand, while the remaining four (nad1, nad4, nad4L, and nad5) were encoded on the L-strand (Additional files 2, 3 4, 5, 6). This transcriptional polarity is identical in all reported decapod mitochondrial genomes. Moreover, they all contained two reading frames overlapped on the same strand: atp6 and atp8, nad4 and nad4L each

![Figure 2](image-url)
shared seven nucleotides. No notable reduction or extension of gene length as compared to other decapods was observed.

In *A. edulis*, *U. major* and *T. kelanang* mitochondrial genomes, lrRNA and srRNA were separated by tRNA^Val^, while the two rRNA genes in *N. glyptocercus* and *N. thermophilus* mtDNA were adjacent to each other (Figure 2). The rRNAs were both coded on L-strand. All five mitochondrial genomes had typical 22 tRNA genes, which ranged from 61 to 73 bp in length (Additional files 2, 3 4, 5, 6), and all of them (except tRNA^Ser^ (AGN)) formed a typical cloverleaf secondary structure. The tRNA^Ser^ (AGN) lacked DHC arm, a feature commonly observed in metazoan mtDNAs [54].

The non-coding regions of *A. edulis*, *U. major*, *T. kelanang*, *N. glyptocercus* and *N. thermophilus* mtDNAs were 845, 1,188, 784, 162, 581 bp, respectively (see Additional files 2, 3 4, 5, 6). Of these regions, the largest non-coding region in each genome was assumed to be the control region (CR) with high A + T composition (Additional file 1). The mtDNA of *N. glyptocercus* had the shortest CR (91 bp) among decapods, and its A + T content was the lowest (59.3%) (Additional file 1). The remaining non-coding regions of the five mitochondrial genomes were considered to be intergenic spacers. Most intergenic spacers contained a few nucleotides (1–56 bp) (Additional file 2, 3 4, 5, 6). However, a relatively large spacer, 177 bp in length, was found between srRNA and tRNA^GI^ in the *U. major* mtDNA (Figure 2 and Additional file 3). Further analyses showed that this large region had an A + T content of 89.8%, higher than that in control region (85.2%).

**Gene order**

The complete genome arrangements of five mud shrimps were depicted in Figures 3 and 4. The gene order of *T. kelanang* mtDNA was identical to that of the pancrustacean (Crustacea + Hexapoda) ground pattern [55], while the genomic organization of four other mud shrimps showed two novel gene orders compared to other mt genomes in the MitoZoa database. Specifically, the mitochondrial genomes of *A. edulis* and *U. major*, and *N. glyptocercus* and *N. thermophilus*, shared the same gene order, respectively.

Compared with the pancrustacean ground pattern, at least five genes were rearranged in each of the mt genome of *A. edulis*, *U. major*, *N. glyptocercus* and *N. thermophilus* (Figures 3 and 4). The tRNA^Leu^ (L1) located between nad1 and lrRNA in other arthropod mtDNAs, was found between tRNA^Leu^ (L1) and cox2 in *A. edulis* and *U. major*, and between cox1 and tRNA^Leu^ (L1) in *N. glyptocercus* and *N. thermophilus*. The tRNA^Asp^ (I) was located between tRNA^Ser^ (UCN) (S3) and nad1 in *A. edulis* and *U. major*, and between srRNA and CR in *N. glyptocercus* and *N. thermophilus*. In *A. edulis* and *U. major*, tRNA^Glu^ (Q) moved upstream to the putative control region, and tRNA^Cys^ (C) and tRNA^Asp^ (Y) moved to the location between CR and tRNA^Mtx^ (M). Additionally, in *N. glyptocercus* and *N. thermophilus* mtDNAs, the tRNA gene tRNA^Val^ (V) changed to downstream of tRNA^Gly^ (G), tRNA^Asp^ (D) moved to upstream of tRNA^Mtx^ (M), and only one protein-coding gene cox3 was involved in the rearrangement. The cox3 located between *atp6* and tRNA^Gly^ (G) in other crustaceans moved upstream to tRNA^Ala^ (A) in *N. glyptocercus* and *N. thermophilus*. All these genes rearranged in the same orientation as the mitochondrial gene arrangement of pancrustacean ground pattern with the exception of tRNA^Leu^ (CLIN) (L1) in the four mud shrimps, and tRNA^Asp^ (I) and tRNA^Val^ (V) in *N. glyptocercus* and *N. thermophilus*. Noticeably, the two tRNA^Leu^ sequences in each mt genome of the four mud shrimps shared significant identity with each other, and the similarity was 86% in *A. edulis*, 80% in *U. major*, 77% in *N. glyptocercus* and 93% in *N. thermophilus*.

**Phylogenetic analysis**

The concatenated alignments of nucleotide and amino acid data from all 13 protein-coding genes were used to investigate the phylogenetic relationships among major lineages of Decapoda. For each dataset, the BI and ML analyses generated nearly identical tree topology except for two branches denoted by open arrowheads, which strongly supported the monophyly of Decapoda (Figure 5A and B). Values of nodal support were typically congruent between the two trees. Both the nucleotide and amino acid phylogenies indicated strong support (BPP/ML bootstrap value in nucleotide phylogeny = 0.99/93, BPP/ML bootstrap value in amino acid phylogeny = 0.99/74) for the separation of two suborders Dendrobranchiata and Pleocyemata in Decapoda. The placing of *Caridea* at the base of *Pleocyemata* was well supported (0.80/93, 0.99/69). The remaining natant decapod *Stenopodidea* was sister with *Reptantia* with a strong support in amino acid phylogeny (BPP/ML bootstrap value = 0.98/79) but only moderate support in nucleotide phylogeny (BPP = 0.70). *Reptantia* was strongly supported to be a monophyletic group (0.99/100, 1.00/100).

Within *Reptantia*, the *Brachyura* and *Anomura* (i.e. *Mieura*) were reciprocally monophyletic (0.99/100, 1.00/100), and their sister relationship also received support (0.71/85, 99/61). The monophyly of *Thalassinidea* (Gebiidea + Axidea) was not supported in the nucleotide phylogeny or the amino acid phylogeny. Yet *AU* test could not reject monophyly of *Thalassinidea*. Gebiidea and Axidea were both shown to be well supported clades (0.99/100, 1.00/100), with moderate support
**Figure 3** Proposed mechanism for the mitochondrial gene arrangement of *Austinogebia edulis*, *Upogebia major* (Decapoda: Gebiidea).

Gene order of *Thalassina kelanang* is identical to that of pancrustaceans ground pattern. Arrows and shaded boxes indicate rearranged genes. Gene segments are not drawn to scale. All genes are transcribed from left to right except for those underlined that exhibit opposite transcriptional orientation.

**Figure 4** Proposed mechanism for the mitochondrial gene arrangement of *Neaixius glyptocercus*, *Nihonotrypaea thermophilus* (Decapoda: Axiidea). Arrows and shaded boxes indicate rearranged genes. The circling arrow indicates inversion. Gene segments are not drawn to scale. All genes are transcribed from left to right except for those underlined that exhibit opposite transcriptional orientation.
(0.66/63, 0.87/-) for the sister relationship between Gebiidea and Mieura. However, the position of Axisidea in Reptantia was incongruent between the nucleotide and amino acid trees. Similarly, there was no support for the monophyly of the lobsters (Achelata + Astacidea).

**Discussion**

**Molecular features of mitochondrial genomes in mud shrimps**

Features of decapod mitochondrial genomes include a high A + T content and rearranged gene structure [56-58]. These features are also apparent in the complete mtDNA sequences of four of the mud shrimps studied, i.e., *A. edulis*, *U. major*, *N. glyptocercus* and *N. thermophilus*. Together with *T. kelanang* mtDNA, all five mitochondrial genomes have the same gene number as other pancrustaceans (13 PCGs, 2 rRNAs, 22 tRNAs). However, the *U. major* mtDNA annotated by Kim et al. [53] has an extra tRNA^Leu(CUN) between nad1 and brRNA. The different annotation is due to ambiguilous beginning of lrRNA and identification of tRNA^Leu(CUN). No anticodon and tRNA-like secondary structure of tRNA^Leu(CUN) is identified between nad1 and lrRNA in the *U. major* mtDNA we sequenced, as in the one sequenced by Kim et al. [53].

Most variations in size in mitochondrial genomes are caused by sequences in non-coding regions [53,58]. *N. glyptocercus* mtDNA has the shortest control region among the decapod mtDNA published, while in *U. major* mtDNA, a relative large intergenic spacer (177 bp) with high A + T content is discovered. Such a large AT-rich region other than CR rarely occurs in malacostracan species and has only been reported in the stomatopods *Oratosquilla oratoria* [59] and *Squilla mantis* [60]. But the length and position of the second AT-rich region are different among the above three species. Moreover, such an AT-rich region is notably absent in the other four mud shrimps, indicating that it is not a conserved feature of thalassinidean mtDNA.

The pancrustacean ground pattern is well retained in *T. kelanang* mitogenome, suggesting that the ancestor of mud shrimps (or at least the Gebiidea) had a typical pancrustacean mtDNA gene order. However, the other four mud shrimps, *A. edulis*, *U. major*, *N. glyptocercus* and *N. thermophilus* have rearranged mitochondrial genomes compared to the pancrustacean ground pattern...
(Figure 3) [56,57,61,62]. Further searches in the MitoZoa database show that mitochondrial sequences of *A. edulis* and *U. major*, *N. glyptocerus* and *N. thermophilus* exhibit two novel genome structures, respectively. Except for tRNA^{Leu(CUN)} in the rearranged genes of *A. edulis* and *U. major* belonged to Gebiidea occur at tRNA^{Ile}-tRNA^{Pyr} junction. However, the rearranged genes of the two axiids *N. glyptocerus* and *N. thermophilus* occur more dispersedly.

**Possible mechanisms for gene rearrangement**

Two major categories of mechanisms have been suggested to explain mitochondrial gene rearrangement: (1) tandem duplication followed by random or non-random deletion [63,64], and (2) non-homologous recombination [65,66]. The first mechanism may explain many or most of the observed rearrangements, while the second one has been proposed to explain gene translocation and inversion [56,67]. Combined the above mechanisms and the results from CREx (Additional files 7 and 8), the rearrangement of four mud shrimps mtDNAs can be depicted as three or four steps (Figures 3 and 4).

For *A. edulis* and *U. major* mtDNA, firstly, transposition of tRNA^{Ile} (I), tRNA^{Cys} (C) and tRNA^{Pyr} (Y) occurred before duplication. If this event occurred after duplication, more genes were duplicated and longer distance translocation were required (Figure 3A). Secondly, an independent duplication/random loss events occurred to account for the translocation of tRNA^{Glu} (Q) (Figure 3B). Thirdly, a duplication/anticodon mutation/non-random loss event [52,68] is expected to account for translocation of tRNA^{Leu(CUN)} (L1) (Figure 3C). A duplication of tRNA^{Leu(UBI)} (L2) on H-strand might have happened. One of the duplicated tRNA^{Leu(UBI)} (L2) changed into tRNA^{Leu(CUN)} (L1) by anticodon mutation. Subsequently the ancestral tRNA^{Leu(CUN)} (L1) lost the function and eventually is deleted from L-strand.

For *N. glyptocerus* and *N. thermophilus* mtDNA, transposition of tRNA^{Ile} (I) occurred first followed by one recombination event (Figure 4A). Then an independent duplication/random loss event occurred to account for the translocation of cox3 (Figure 4B). This was followed by transposition of tRNA^{Asp} (D) and tRNA^{Val} (V) and a recombination event (Figure 4C). Finally, a duplication/anticodon mutation/non-random loss event [52,68] occurred to account for the translocation of tRNA^{Leu(CUN)} (L1) (Figure 4D).

Another possibility for the translocation of tRNA^{Leu(CUN)} (L1) predicted by CREx is based on “duplication/random loss model” [63]. This interpretation seems less likely given that there is a long distance between tRNA^{Leu(UBI)} (L2) and tRNA^{Leu(CUN)} (L1) in the ancient arrangement, as well as the presence of inversion in tRNA^{Leu(CUN)} (L1) [52]. Moreover, the sequence homology between tRNA^{Leu(UBI)} (L2) and tRNA^{Leu(CUN)} (L1) in *A. edulis, U. major, N. glyptocerus* and *N. thermophilus* is higher than any two other randomly chosen tRNAs. It seems to be more possible that two tRNA^{Leu} arose from duplication followed by anticodon mutation. The similar duplication/anticodon mutation events have also been reported in other crustaceans, for example in amphipods *Caprella scaura* [69] and *Gammarus duebeni* [70], and decapods *Geothelphusa dehaani* [71] and *Stenopus hispidus* [52].

Under the above models, including the random and non-random loss, incomplete deletion or partial retention of duplication resulted in the formation of the multiple intergenic spacers (Additional files 2, 3, 4, 5, 6). These results indicate that intergenic spacers might serve as a guide in deducing the generation of gene rearrangement [56]. Moreover, the distinct rearrangement processes suggest that Gebiidea and Axiidea evolved independently from the pancrustacean ground pattern.

**Phylogenetic relationships of the major clades in Decapoda**

With higher taxon samplings and the inclusion of all the major groups of decapod crustaceans, the present complete mitochondrial genomic analysis strongly supports that the Caridea is sister to the other Pleocyemata. Similar to the results of Shi et al. [52], Stenopodidae is revealed to be a sister clade of Reptantia. While this relationship is only weakly supported in Shi et al. [52], this grouping is strongly supported in our tree based on amino acid sequences.

Within the Reptantia, the sister relationship between Brachyura and Anomura (i.e. the Meiura) has always received very high support in complete mitochondrial genomic analyses [51,52,62]. The present result suggests that Gebiidea is the sister group of Meiura though only with moderate support. In general, the topology of the currently most extensive complete mitochondrial genomic tree of decapod crustaceans (particularly the one based on amino acid sequences) is most similar to the most recent mt genome analyses by Kim, Park, et al. [51], and those of Scholtz and Richter [10] and Ahyong and O’Meally [15] deduced from morphology and combined morphology and molecular (16S and 28S) data, respectively. The trees of Scholtz and Richter [10] and Ahyong and O’Meally [15] are essentially the same except for the identity of the sister clade of Thalassinidea (i.e. Gebiidea + Axiidea), which is considered to be monophyletic. Thalassinidea is sister to Meiura in Ahyong and O’Meally [15] but in Scholtz and Richter [10] it is sister to the clawed lobster Astacidea which is shown to be polyphyletic. The main difference between the present result with these two analyses is that Gebiidea and Axiidea do not make up a monophyletic group while the position of Astacidea is unresolved. The
mitogenome tree of Kim et al. [50] includes only one mud shrimp species belonged to Gebiidea. Adding two more species and genera of Gebiidea in the present work reveals a similar topology of Gebiidea being sister to Mieura. However, the other mud shrimp group Axiidea does not cluster with Gebiidea.

As the results of complete mitochondrial genome sequence analysis are now rather consistent with the conclusions deduced from some morphology and partial gene sequence data, it seems to be promising in using complete mtDNA sequence to reconstruct the evolutionary history of decapod crustaceans. Nevertheless, more taxon sampling, particularly the inclusion of certain key groups such as the sargassid shrimp (supposed to be sister to Penaeoidea in Dendrobranchiata), blind lobsters Polycheilida [4], primitive cave shrimp Procracididea [7], the living fossil lobster Glyphidea [23], the enigmatic shrimp Luciferidae [72] and the various bizarre anomuran groups [73], will be necessary to achieve this goal.

Conclusions
This study presents five complete mitochondrial genomes of mud shrimps, Austinoegibia edulis, Upogebia major and Thalassina kelanang of Gebiidea, and Niphonotrypaea thermophilus and Neaxius glyptocercus of Axiidea. The contents of individual mt genes in these five mud shrimps are similar to that in other decapods. The U. major mt genome contain a relative large intergenic spacer with higher A + T content than that in control region. The N. glyptocercus mt genome, the shortest decapod mtDNA known, has the shortest control region. Except for T. kelanang, the other four mud shrimps have rearranged mt genomes compared to pancrustacean ground pattern. A duplication/loss (random and nonrandom) and recombination model may result in their mt gene order. The different gene arrangement process suggests the derived gene orders of Gebiidea and Axiidea might have evolved independently. Phylogenetic analyses do not support the monophyly of mud shrimps, while the positions of Gebiidea and Axiidea in Reptantia are poorly resolved.

Methods
Sample collection and DNA extraction
The collecting sites of the mud shrimp specimens used in the present study are A. edulis from Starfish Bay, Hong Kong, U. major from Qingdao No.1 Bathing Beach, China, T. kelanang from Kelanang Beach, Selangor, Malaysia, N. glyptocercus from Kenting, Taiwan and N. thermophilus from Kueishan Island, Taiwan. The specimens obtained were stored in 75-95% ethanol. Total genomic DNA for all species was extracted from tissues by using a DNeasy tissue kit (Qiagen) following the manufacturer’s protocol.

PCR and sequence determination of A. edulis and U. major mitochondrial genomes
Four short fragments of the genes cox1, nad5, lrrna and cob were first determined by PCR with the universal primer sets of LCO1490/HCO2198 [74], nad5F/nad5R [75], 16S1471/16S1472 [76] and cobF424/cobR876 [77]. PCR products were purified using the Qiiaquick Gel extraction Kit (Qiagen) and directly sequenced with ABI 3730xl DNA Analyzer.

Based on the sequences obtained above, long PCR primers were designed to amplify the entire A. edulis (AE) and U. major (UM) mitochondrial genomes in four fragments: AE/UMcox1F-AE/UMnad5R, AE/UMnad5F-UE/UMcobR, and UE/UMcobF-AE/UM16SR, AE/UM16SR-AE/UMcox1R, with the PCR products of approximate 5.5 kb, 3.7 kb, 1.9 and 4.5 kb in length, respectively (Additional file 9). PCR reactions were carried out in 25 μl reaction mixtures containing 18.8 μl of sterile distilled H2O, 2.5 μl of 10× LA PCR buffer II (Mg2+ plus, Takara), 0.5 μl of dNTP (10 mM each), 1 μl of each primer (5 μM), 0.2 μl of LA Taq polymerase (5 unit/μl, Takara), and 1 μl of DNA template (approximate 30 ng). The amplifications were performed on TaKaRa PCR Thermal Cycler Dice Model TP600 (Takara Bio Inc.) with an initial denaturation at 94° for 3 min, followed by 34 cycles of denaturation at 94° for 20 s, annealing at 50-52° for 50 s, extension at 68° for for 1 min/kb, and a final extension at 68° for 10 min. Long PCR products were purified using the Qiaquick Gel extraction Kit (Qiagen) and bidirectionally sequenced using a primer-walking strategy on ABI 3730xl DNA Analyzer.

PCR and sequence determination of T. kelanang, N. glyptocercus and N. thermophilus mitochondrial genomes
Four partial fragments of the genes cob, cox1-2cox2 (2 kb), cox3, srRNA were first determined by PCR with the primer sets of Cyb1/Cyb2 [78] crust-couxIF [79]/CCO2Rv1 [80], Scox3-F(GGCCCTTCAGTNGAAAATTGG)/Scox3-R (ACTA CATCDAACRAATGTCAATATCA), and srRNA-F (AAAT TTAAATTCAACATCGAGGTCGCAAAACT)/srRNA-R (TT GACYGTCRCAAGGTAAGCATATAATAG). Additional three partial mitochondrial sequences (nad4, nad5 and 12S) of T. kelanang were also determined with PCR primer sets of L11424-ND4/H11534-ND4M [81], crust-nd5R/crust-nd5F and crust-12Sf/crust-12Sr [79].

Based on the sequences obtained above, long PCR primers were designed to amplify the entire T. kelanang (TK), N. glyptocercus (NG) and N. thermophilus (NT) mitochondrial genomes in several fragments: TKbs-R/H11534-ND4M [81], TKc1s-R/TK12s-R, TKc2s-F/TKd5s-R, NGbs-f/NGRs-R, NGcs-f/NGbs-R, NGs
and amino acid datasets consisted of 7,577 and 3,781 sites, respectively. Phylogenetic trees were built by two approaches including Bayesian inference (BI) analysis using Phylobayes 3.3b [92] and maximum-likelihood (ML) analysis using RaXML 7.0.4 [93].

For the nucleotide dataset, the model GTR + I + G was selected by JMODELTEST 0.11 [94]. The model MtRev + I + G + F was chosen as the best-fit model for the amino acid dataset by ProtTest version 2.4 [95]. According to preliminary analysis, the categories model with GTR (CAT-GTR) and CAT-Poisson models [96,97] fit the data best and were used for BI and ML analysis of the nucleotide and amino acid data, respectively. For BI analysis, two independent MCMC chains were run simultaneously to determine whether the searching reached stabilization, and were stopped when all chains converged (maxdiff less than 0.1). For ML analysis, 1000 bootstraps were used to estimate the node reliability. Topology testing was performed using Consel [98] for the approximately unbiased (AU) test [99].

**Additional files**

- **Additional file 1:** Genomic characteristics of decapod mitochondrial genomes.
- **Additional file 2:** Location of genes in the mitochondrial genome of Austinogebia edulis.
- **Additional file 3:** Location of genes in the mitochondrial genome of Upogebia major.
- **Additional file 4:** Location of genes in the mitochondrial genome of Thalassina kelanang.
- **Additional file 5:** Location of genes in the mitochondrial genome of Neaxius glyptocercus.
- **Additional file 6:** Location of genes in the mitochondrial genome of Nihonotrypaea thermophilus.
- **Additional file 7:** Mitochondrial gene order rearrangement scenario of Austinogebia edulis, Upogebia major (Decapoda: Gebiidea) inferred by CREx. The elements in the blue shaded boxes are lost in the second copy, therefore the remaining copies are moved to the front. The elements in the red shaded boxes are lost in the first copy, therefore the remaining copies are moved to the back.
- **Additional file 8:** Mitochondrial gene order rearrangement scenario of Neaxius glyptocercus and Nihonotrypaea thermophilus (Decapoda: Axidea) inferred by CREx. The elements in the blue shaded boxes are lost in the second copy, therefore the remaining copies are moved to the front. The elements in the red shaded boxes are lost in the first copy, therefore the remaining copies are moved to the back.
- **Additional file 9:** Specific primers used in amplification of Austinogebia edulis and Upogebia major mitochondrial genomes.
- **Additional file 10:** Specific primers used to amplification of the fragments which covered the gaps after sequencing Thalassina kelanang, Neaxius glyptocercus and Nihonotrypaea thermophilus mitochondrial genomes.

**Abbreviations**

- atp6 and 8: ATPase subunits 6 and 8; bp: Base pair(s); BI: Bayesian inference; BP: Bootstrap, cox1-3: Cytochrome c oxidase subunits I-III; cob: Cytochrome b; lrRNA: 16S ribosomal RNA; ML: Maximum likelihood; Mt: Mitochondrial; mtDNA: Mitochondrial DNA; mt: Mitochondrial; nd: NADH dehydrogenase subunits 1-6 and 4L; ORF: Open reading frame; PCG: Protein...
coding gene; PCR: Polymerase chain reaction; BPP: Bayesian posterior probabilities; rRNA: Ribosomal RNA; sRNA: 12S ribosomal RNA; tRNA: Transfer RNA; tRNA gene: tmX (where X is replaced by single letter amino acid code of the corresponding amino acid).

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TYC, KHC and ZC contributed to the conception and design of the study. FJL and YL performed the laboratory works of TYC, KHC and ZC contributed to the conception and design of the study. FJL and YL cooperated with the writing of the manuscript. ZC supervised the study and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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Author details
1. Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan. *EMBL, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China. 2. Simon F. S. Li Marine Science Laboratory, School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong. 3. Institute of Marine Biology and Center of Excellence for Marine Bioenvironment and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan.

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Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TYC, KHC and ZC contributed to the conception and design of the study. FJL and YL performed the laboratory works of A. adulus, T. kelsonii, N. glyptocercus and N. thermophilus. ZS and RL conducted the work on U. yuatan. YL and LMT performed bioinformatics analyses of nucleotide and protein sequences. FJL, ML and YL cooperated with the writing of the manuscript. ZC supervised the study and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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