Digging deeper: New gene order rearrangements and distinct patterns of codons usage in mitochondrial genomes among shrimps from the Axiidea, Gebiidea and Caridea (Crustacea: Decapoda)

Mun Hua Tan [1,2] , **Han Ming Gan** [Corresp., 1, 2] , **Yin Peng Lee** [1,2] , **Gary C. B. Poore** [3] , **Christopher M Austin** [1,2,4]

[1] School of Science, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

[2] Genomics Facility, Tropical Medicine and Biology Platform, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

[3] Museum of Victoria, Melbourne, Victoria, Australia

[4] School of Life and Environmental Sciences, Deakin University, Burwood, Australia

Corresponding Author: Han Ming Gan
Email address: gan.han.ming@monash.edu

Background. Whole mitochondrial DNA is being increasingly utilized for comparative genomic and phylogenetic studies at deep and shallow evolutionary levels for a range of taxonomic groups. Although mitogenome sequences are deposited at an increasing rate into public databases, their taxonomic representation is unequal across major taxonomic groups. In the case of decapod crustaceans, several infraorders, including Axiidea (ghost shrimps, sponge shrimps, mud lobsters) and Caridea (true shrimps), are still under-represented, limiting comprehensive phylogenetic studies that utilize mitogenomic information.

Methods. Sequence reads from partial genome scans were generated using the Illumina MiSeq platform and mitogenome sequences were assembled from these low coverage reads. In addition to examine phylogenetic relationships within the three infraorders, Axiidea, Gebiidea and Caridea, we also investigated the diversity and frequency of codon usage bias and mitogenome gene order rearrangements.

Results. We present new mitogenome sequences for five shrimp species from Australia that includes two ghost shrimps, *Callianassa ceramica* and *Trypaea australiensis*, along with three caridean shrimps, *Macrobrachium bullatum*, *Alpheus lobidens* and *Caridina* cf. *nilotica*. Strong differences in codon usage were discovered among the three infraorders and significant gene order rearrangements were observed. While the gene order rearrangements are congruent with the inferred phylogenetic relationships and consistent with taxonomic classification, they are unevenly distributed within and among the three infraorders.

Discussion. Our findings suggest potential for mitogenome rearrangements to be useful phylogenetic markers for decapod crustaceans and at the same time raise important questions concerning the drivers of mitogenome evolution in different decapod crustacean lineages.

1 **Digging deeper: New gene order rearrangements and distinct patterns of codons usage in**

2 **mitochondrial genomes among shrimps from the Axiidea, Gebiidea and**

3 **Caridea (Crustacea: Decapoda)**

5 Mun Hua Tan[1,2], Han Ming Gan[1,2], Yin Peng Lee[1,2], Gary C. B. Poore[3] and Christopher M.

6 Austin[1,2,4]

9 [1] School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway 47500,

10 Petaling Jaya, Selangor, Malaysia

11 [2] Genomics Facility, Tropical Medicine and Biology Platform, Monash University Malaysia,

12 Jalan Lagoon Selatan, Bandar Sunway 47500, Petaling Jaya, Selangor, Malaysia

13 [3] Museum Victoria, PO Box 666, Melbourne, Victoria, Australia

14 [4] School of Life and Environmental Sciences, Deakin University, Burwood, Victoria 3125,

15 Australia

18 **Corresponding author:**

19 Han Ming Gan[1,2]

21 Email address: gan.han.ming@monash.edu

32 **Abstract**

34 **Background.** Whole mitochondrial DNA is being increasingly utilized for comparative genomic

35 and phylogenetic studies at deep and shallow evolutionary levels for a range of taxonomic

36 groups. Although mitogenome sequences are deposited at an increasing rate into public

37 databases, their taxonomic representation is unequal across major taxonomic groups. In the case

38 of decapod crustaceans, several infraorders, including Axiidea (ghost shrimps, sponge shrimps,

39 mud lobsters) and Caridea (true shrimps), are still under-represented, limiting comprehensive

40 phylogenetic studies that utilize mitogenomic information.

41

42 **Methods.** Sequence reads from partial genome scans were generated using the Illumina MiSeq

43 platform and mitogenome sequences were assembled from these low coverage reads. In addition

44 to examine phylogenetic relationships within the three infraorders, Axiidea, Gebiidea and

45 Caridea, we also investigated the diversity and frequency of codon usage bias and mitogenome

46 gene order rearrangements.

47

48 **Results.** We present new mitogenome sequences for five shrimp species from Australia that

49 includes two ghost shrimps, *Callianassa ceramica* and *Trypaea australiensis*, along with three

50 caridean shrimps, *Macrobrachium bullatum*, *Alpheus lobidens* and *Caridina* cf. *nilotica*. Strong

51 differences in codon usage were discovered among the three infraorders and significant gene

52 order rearrangements were observed. While the gene order rearrangements are congruent with

53 the inferred phylogenetic relationships and consistent with taxonomic classification, they are

54 unevenly distributed within and among the three infraorders.

55

56 **Discussion.** Our findings suggest potential for mitogenome rearrangements to be useful

57 phylogenetic markers for decapod crustaceans and at the same time raise important questions

58 concerning the drivers of mitogenome evolution in different decapod crustacean lineages.

59

60 **Introduction**

61   Sequencing of animal mitochondrial genomes has exploded in recent years. Over 40,000

62 animal mitogenomes are currently lodged on the NCBI database compared to fewer than 5,000

63   sequences a decade ago. As mitogenomic data have accumulated it has become apparent that

64   mitochondrial gene order is not as conserved as first thought, and that interesting and

65   phylogenetically-useful patterns that invite further research are emerging (Boore 2006; Dowton

66   et al. 2002; Gan et al. 2014a; Gissi et al. 2008; Lin et al. 2012; Poulsen et al. 2013; Tan et al.

67   2015).

68   In addition to gene order evolution, properties of mitogenome sequences that are of

69   phylogenetic significance have emerged that are of interest to comparative mitogenomics such as

70   gene loss and duplications, AT bias, strand asymmetry in nucleotide composition, length and

71   structure of the control region, features of intergenic non-coding regions, codon usage, variation

72   in gene length, variation in start and stop codons, gene diversity levels, mutation rates and

73   signals of selection and secondary structures of ribosomal genes (Bauzà-Ribot et al. 2009; Gissi

74   et al. 2008; Jia & Higgs 2008; Li et al. 2015; Oliveira et al. 2008; Poulsen et al. 2013; Qian et al.

75   2011; Shoemaker et al. 2004). However, despite the rapidly accumulating mitogenomic

76   resources, there are gaps in taxonomic representation and more data are required to fully evaluate

77   the usefulness of mitogenome gene rearrangements as phylogenetic markers in specific groups

78   (Mao et al. 2014; Tan et al. 2015) and for broadly-based comparative studies to detect patterns

79   and investigate evolutionary hypotheses (Boore 2006; Castellana et al. 2011; Gissi et al. 2008;

80   Jiang et al. 2007).

81   In general, the overall structure and function of animal mitochondrial genomes are

82   remarkably stable. It is a circular, double-stranded DNA molecule of usually 15-20 kb in length,

83   generally containing a consistent set of 37 genes made up of two ribosomal RNA genes (rRNA),

84   13 protein-coding genes (PCG) and 22 transfer RNA genes (tRNA) (Bernt et al. 2013; Castellana

85   et al. 2011; Gissi et al. 2008). Intergenic regions are usually minimal, although all species

86   contain at least one large AT-rich region associated with strand replication e.g. the control region.

87   Mutations in the mitochondrial DNA can range from point mutations and infrequent

88   insertions/deletions to gene order rearrangements. However, the type, scale and distribution of

89   mutations can vary widely across various taxonomic levels (Gissi et al. 2008; Tan et al. 2015).

90   The most common mitogenome gene order rearrangements involve the translocation of

91   single tRNA genes and occasionally with a change of transcriptional polarity or duplication. Less

92   frequent is the repositioning of multiple tRNAs, duplication of the control region, or changes to

93   the order and orientation of protein coding and rRNA genes. The paradox of mitogenome gene

94 rearrangements is that the molecule can be highly conserved among phylogenetically-distant

95 species such as some insect and decapod crustacean species but can, in a restricted number of

96 taxonomic groups, also vary substantially among species in the same family or genus. Examples

97 of taxa with a concentration of reported mitogenome gene rearrangements include ticks (Fahrein

98 et al. 2007; Shao et al. 2005), hymenopterans (Dowton et al. 2003), gulper eels (Poulsen et al.

99 2013), salamanders (Chong & Mueller 2013), tunicates (Gissi et al. 2004), and in several

100 crustacean groups (Kilpert & Podsiadlowski 2006; Kim et al. 2012; Miller et al. 2004; Stokkan et

101 al. 2016; Tan et al. 2015).

102 Codon usage is another aspect of comparative mitogenomics that is attracting increasing

103 attention as research moves from describing patterns to understanding them within an

104 evolutionary and molecular genetic context (Castellana et al. 2011; Gissi et al. 2008; Jiang et al.

105 2007). Amino acids can be encoded by two to six codons, but alternative codons for the same

106 amino acid often do not occur at equal frequencies either between species for the same gene or

107 between different genes in the one species. Patterns of differential codon usage have been

108 attributable to selection, variable mutation rates, translational efficiency and random factors

109 (genetic drift) (Castellana et al. 2011; Jia & Higgs 2008; Prat et al. 2009; Whittle & Extavour

110 2015). However, it has been rarely addressed among crustacean species (Cook et al. 2005;

111 García-Machado et al. 1999; Rota-Stabelli et al. 2013).

112 As in other major animal groups, mitogenome sequences are becoming increasingly

113 available for decapod crustacean species, contributing to the understanding of the evolution of

114 this taxonomically challenging group due to its high diversity, deep lineages and highly flexible

115 body plan (Shen et al. 2013; Tan et al. 2015). In addition, intriguing and taxonomically unevenly

116 distributed gene order rearrangements are emerging, requiring further investigation and raising

117 questions regarding the dynamics and drivers of mitogenome gene order evolution in several

118 groups (Gan et al. 2015; Kilpert & Podsiadlowski 2006; Miller et al. 2004). Two of the less well-

119 represented decapod crustacean infraorders in mitochondrial databases are the shrimp infraorders

120 Axiidea (ghost shrimps, sponge shrimps, mud lobsters) and Caridea (true shrimps) (Lin et al.

121 2012; Tan et al. 2015). Further, the phylogenetic relationships within and among these shrimp

122 groups remain largely unresolved and disputed (Lin et al. 2012; Timm & Bracken-Grissom

123 2015). A major limiting factor to the resolution of phylogenetic relationships within and among

124 these shrimp lineages and the determination of the distribution and evolutionary significance of

125 mitogenome gene order rearrangements is inadequate taxon sampling (Lin et al. 2012; Shen et al.

126 2013; Tan et al. 2015).

127   At the present time, the Axiidea, Gebiidea and Caridea are represented by only six, five

128 and 17 complete mitogenomes respectively on the NCBI public database. To support ongoing

129 phylogenetic and comparative mitogenomic studies, this paper reports five new mitogenome

130 sequences of shrimp species sampled from Australia. These include two mitogenomes from the

131 ghost shrimp, *Callianassa ceramica* Fulton & Grant, 1906 and *Trypaea australiensis* Dana, 1852

132 (Callianassidae), and three from caridean shrimps *Macrobrachium bullatum* Fincham, 1987

133 (Paleamonidae), *Alpheus lobidens* De Haan, 1849 (Alpheidae) and *Caridina* cf. *nilotica* Roux,

134 1833 (Atyidae), each of which represents highly diverse superfamilies, families and genera

135 within Caridea. This study compares the mitogenomic features of these five species together with

136 additional representatives of their infraorders and Gebiidea available from the NCBI database. In

137 addition to exploring evolutionary relationships within each infraorder, we uncover distinctive

138 signatures and patterns with respect to sequence composition, codon usage bias and gene

139 rearrangements that can possibly act as synapomorphies for specific shrimp taxonomic groups,

140 suggesting the potential of these features for phylogenetic inferences at different evolutionary

141 scales.

142

143 **Materials and Methods**

144

145 *Sample collection*

146

147   Two species belonging to the infraorder Axiidea (*Callianassa ceramica*, *Trypaea*

148 *australiensis*) and three from Caridea (*Macrobrachium bullatum*, *Alpheus lobidens*, *Caridina* cf.

149 *nilotica*) were collected from different locations in Australia (Table 1). *Callianassa ceramica* is

150 represented by two individuals: one from a vouchered specimen (Museum Victoria J40715;

151 GenBank accession number KU350630.1); and the other a previously sequenced sample

152 published incorrectly as *T. australiensis* (KM501040.1) (Gan et al. 2014b). The mitogenome

153 sequence for this latter sample is now registered as *C. ceramica* under the accession number

154 KU726823.1 and has a genetic similarity of 99.8% to the COI region of a vouchered *C. ceramica*

155 specimen (Museum Victoria J70519) collected from the same general locality.

156   To maintain continuity with the original NCBI accession number and species name, the

157   original mitogenome sequence lodged on NCBI for *T. australiensis* was updated with the newly-

158   sequenced mitogenome from a vouchered *T. australiensis* specimen (Museum Victoria J40711;

159   GenBank accession number: KM501040.2). The accession numbers for each species and

160   associated collecting and identification-related information are detailed in Table 1 and Data S3,

161   including voucher numbers for specimens lodged in in Museum Victoria, Melbourne (NMV) and

162   the Museum and Art Gallery of the Northern Territory, Darwin (MAGNT).

163

164   Next-generation sequencing and mitogenome assembly

165

166   Purification of ethanol-preserved tissue and partial whole genome sequencing (2×75 bp

167   for *T. australiensis* and 2×250 bp for others) was performed on the Illumina MiSeq platform as

168   previously described (Gan et al. 2014a), after which each mitogenome was assembled with

169   IDBA_UD v.1.1.1 (Peng et al. 2012) and annotated using MITOS (Bernt et al. 2013). Circular

170   mitogenome maps were drawn with BRIG v.0.9.5 (Alikhan et al. 2011). Summary statistics

171   including gene boundaries and length, strand, nucleotide composition, intergenic nucleotides,

172   and number of genes were compiled with MitoPhAST v.1.0 (Tan et al. 2015). Alignment of

173   whole mitogenome sequences and calculation of pair-wise nucleotide identities were performed

174   with SDT v.1.2 (Muhire et al. 2014).

175

176   *Gene order analysis*

177

178   Along with the five mitogenomes sequenced in this study, sequences from 28 other

179   complete mitogenomes from the three infraorders were obtained from NCBI's RefSeq database

180   (Table 2) for comparative analyses. Arrangements of genes for each of these 33 mitogenomes

181   were compared with all other existing decapod mitogenomes in RefSeq to identify potential

182   novel gene orders unreported by previous studies. Mitogenomes that exhibit gene orders

183   differing from that of the pancrustacean ground pattern (Boore et al. 1998) were re-annotated

184   with MITOS (Bernt et al. 2013) to confirm that differences observed are not due to mis-

185   annotations. Any observed mis-annotations (e.g. missing genes, incorrect gene boundaries) were

186   corrected before proceeding to further comparative and phylogenetic analyses.

187

188   *Phylogenetic analysis*

189

190   Mitogenomes listed in Table 2 were subject to phylogenetic analysis to establish the

191   evolutionary relationships of species within each of the infraorders to provide a framework for

192   interpreting mitogenome gene rearrangements. MitoPhAST v.1.0 (Tan et al. 2015) was used to

193   extract individual PCG amino acid sequences, and these protein sequences were then separately

194   aligned with MAFFT v.7.222 (Katoh & Standley 2013), followed by trimming with trimAl v.1.4

195   (Capella-Gutiérrez et al. 2009). For nucleotide level analyses, PCG nucleotide sequences were

196   manually extracted and fed to TranslatorX v.1.1 (Abascal et al. 2010), which aligns nucleotide

197   sequences guided by amino acid translations and then trimmed with Gblocks v.0.19b (Castresana

198   2000). On the other hand, rRNAwere aligned with MAFFT v.7.222 (*mafft-linsi*) (Katoh &

199   Standley 2013) and trimmed with trimAl v.1.4 (Capella-Gutiérrez et al. 2009). Finally,

200   mitochondrial PCG and rRNA sequences were concatenated into super-alignments to make up

201   the following datasets:

202

203   A. 13 PCG (aa) [3,591 characters]

204   B. 13 PCG (nt) [9,642 characters]

205   C. 13 PCG (aa) + 12S rRNA + 16S rRNA [5,694 characters]

206   D. 13 PCG (nt) + 12S rRNA + 16S rRNA [11,755 characters]

207

208   Maximum-likelihood (ML) tree inference with ultrafast bootstrap (UFBoot) branch

209   supports (Minh et al. 2013) was performed using IQ-TREE v.1.5.0 (Nguyen et al. 2015), which

210   also implements model selection to find the best-fit partitioning scheme. Super-alignments for all

211   datasets were partitioned based on genes. An additional analysis for Dataset B that further

212   partitions it according to 1st, 2nd and 3rd codon positions was also performed. For Bayesian

213   inference, the same super-alignments generated from all datasets were analyzed using Exabayes

214   v.1.4.2 (Aberer et al. 2014). For each analysis, four independent runs were carried out

215   concurrently for 5 million iterations each with 25% of initial samples discarded as burn-in.

216   Convergence of chains was checked by ensuring the average standard deviation of split

217   frequencies (*asdsf*) is below 0.5%, considered to be good convergence according to the Exabayes

218   user guide. Alignments, partitions and best-fit partitioning schemes for all datasets are available

219   as Data S1.

220

221   *Codon usage analysis*

222

223   Codon usage (in counts) was calculated using EMBOSS v.6.5.7 (Rice et al. 2000)

224   followed by minor adjustments based on the Invertebrate Mitochondrial Code (genetic code = 5).

225   Comparisons among the three lineages of shrimps were made by applying the chi-square test to

226   the pooled codon usage counts for species from each infraorder. Relative Synonymous Codon

227   Usage (RSCU) values were calculated by taking the ratio of the number of times a codon appears

228   to the expected frequency of the codon if all synonymous codons for a same amino acid are used

229   equally (Sharp & Li 1987). Patterns of variation among individuals in RSCU values were

230   summarized using multidimensional scaling (MDS) based on Euclidean dissimilarities

231   implemented in XLSTAT v.2015.4.01.20978 (Addinsoft 2010).

232

233   **Results**

234   *Mitogenome composition*

235

236   Mitogenomes for specimen J40715 of *C. ceramica* (16,899 bp, 130× cov), *T.*

237   *australiensis* (15,485 bp, 86× cov), *M. bullatum* (15,774 bp, 27× cov), *A. lobidens* (15,735 bp,

238   60× cov) and *C.* cf. *nilotica* (15,497 bp, 63× cov) were assembled into complete circular

239   sequences, annotated (Fig. 1), and deposited in GenBank with accession numbers listed in Table

240   1. Four of the mitogenomes contain the typical 13 protein-coding genes, two ribosomal RNA

241   genes, 22 transfer RNA genes and one long non-coding region while *A. lobidens* has an

242   additional *trnQ* flanked by the *ND4L* and *trnT* genes (Fig. 1). Detailed composition of each

243   mitogenome can be found in Table S1 while general information on % AT and lengths for all

244   mitogenomes included in this study are in Table S2. The mitogenomes are AT rich (58.9 -

245   69.7%), with *A. lobidens* having the lowest AT content, matching closely to *A. distinguendus*

246   (60.2%), the only other species of *Alpheus* having a published mitogenome sequence. Gene

247   lengths are typical but *Callianassa* and *Trypaea* have an elevated proportion of intergenic

248   nucleotides, with some spacers in the order of 200 bp in length. This is significantly larger than

249   for other members of the Axiidea, but similar to spacers reported for the Gebiidea. *Callianassa*

250   *ceramica* (KU350630.1) has an unusually long control region of 2,036 bp, whereas for all the

251   other taxa it is less than 1,000 bp, including the closely-related *T. australiensis* (587 bp).

252   However, the control region for the conspecific *C. ceramica* (KU362925.1) is very similar

253   (1,978 bp) and these two specimens are also very similar in terms of % AT (69.7% and 70.2%).

254   A matrix of pair-wise identities of whole mitogenome sequence alignments can be found in Fig.

255   S1.

256

257   *Manual inspection and MITOS annotation identified multiple erroneously annotated crustacean*

258   *mitogenomes in GenBank RefSeq database*

259

260   The re-annotated species with gene orders divergent to that of the pancrustacean ground

261   patterns obtained from GenBank's RefSeq database identified several that require revision. One

262   was found to have a missing protein-coding gene and an extra trnS, and others inverted rRNA

263   and tRNA coordinates and other annotation anomalies as detailed in Table 3. For our study,

264   entries were edited based on MITOS annotations and the revised GenBank files for these entries

265   are included as Data S2.

266

267   *Whole mitogenomes are consistent with monophyly of infraorders Axiidea, Gebiidea and*

268   *Caridea*

269

270   A total of 33 mitogenome sequences from the three groups of interest were utilized

271   (Axiidea: 8, Gebiidea: 5, Caridea: 20) to elucidate phylogenetic relationships, with an additional

272   12 Dendrobranchiata mitogenomes as outgroup (Table 2). Trees constructed from every dataset

273   and analysis are available in Fig. S2. All trees place *M. bullatum*, *A. lobidens* and *C.* cf. *nilotica*

274   as sister taxa to other species from their respective genera with relatively high nodal support

275   (UFBoot ≥ 93%, PP 1.00). These trees also consistently place *C. ceramica* as sister to *T.*

276   *australiensis* (UFBoot 100%, PP 1.00) within Axiidea.

277   The Bayesian-inferred phylogenetic tree in Fig. 2A, constructed based on amino acid

278   sequences of 13 PCGs and nucleotide sequences of 2 rRNAs (Dataset C), shows a tree topology

279   that is shared by most trees inferred in this study. Most nodes received maximal support from

280   each analysis and dataset. The greatest levels of uncertainty in terms of phylogenetic placement

281   are mostly relating to the relationships among closely-related taxa, such as in the *Palaemon* (6/10

282   trees), *Rimicaris-Opaepele-Alvinocaris-Nautilocaris* (2/10 trees), *Upogebia-Austinogebia* (1/10

283   trees), *Corallianassa-Paraglypturus* (1/10 trees) and *Macrobrachium* (1/10 trees) clades. The

284   only deeper clade with low support is the placement of Atyidae as the sister clade of

285   Alvinocarididae within Caridea (UFBoot ≥ 90, PP 1.00). Within Axiidea, maximal support is

286   observed for almost all nodes. The most basal split in this infraorder separates Strahlaxiidae

287   *(Neaxius glyptocerus)* from Callianassidae. Within Callianassidae, the three major lineages

288   correspond to accepted subfamilies, two represented by one species each and Callianassinae by

289   four species. In as far as it goes, the phylogenetic placements of mitogenome sequences in

290   Axiidea are congruent with the current classification at the family, subfamily and generic levels

291   (Felder & Robles 2009) (Fig. 2A). These analyses of just four upogebiid species indicate that

292   *Upogebia* may be paraphyletic with respect to *Austinogebia*. Since these two genera are

293   nominally represented by over 120 and eight species respectively, any comment on their status is

294   premature at this stage. The degree of divergence between the species of *Rimicaris* and *Opaepele*

295   is small relative to the degree of divergence between congeneric species within *Macrobrachium*,

296   *Alpheus*, *Caridina* and *Alvinocaris*.

297

298   *Deviation from the pancrustacean ground pattern is prevalent in the currently sequenced*

299   *members of the Axiidea and Gebiidea*

300

301   Most mitogenomes from caridean species have the pancrustacean ground pattern (pattern

302   *Gr*, Fig. 2B). Those that do differ show only minor rearrangements involving the short tRNA

303   genes (patterns *Pa*, *Ap1* and *Ap2*). In contrast, the mitogenomes of species within Axiidea and

304   Gebiidea exhibit relatively substantial differences in gene order (patterns *Up*, *Ax1* and *Ax2*)

305   entailing rearrangements of protein-coding genes, rRNAs and a number of tRNAs. An example

306   is pattern *Ax2* shown in Fig. 2B, which includes the inversion of the *ND1*, *lrRNA, srRNA* and *trnI*

307   genes as a block, in addition to the inversion and translocation of *trnD* from between *trnQ* and

308   *trnM* to a position between *trnS2* and the putative control region, as well as new placements for

309   *trnL* and *trnV* also evident in pattern *Ax1*.

310   All gene order novelties relative to the pancrustacean ground pattern are consistent with

311   the relationships depicted by the molecular phylogeny, and in several cases define taxonomic

312   groups at different levels. In Caridea, pattern *Pa* is common to the three *Palaemon* species and

313   pattern *Ap1* defines the two *Alpheus* species. Similarly, within Gebiidea, while *Thalassina*

314   *kelanang* (family Thalassinidae) has the ground pancrustacean pattern, the other four species are

315   all members of the family Upogebiidae and are united by novel rearrangements involving several

316   tRNA translocations (pattern *Up*, Fig 2B). Pattern *Ax1*, involving the rearrangements of *COIII*

317   and several tRNAs, is shared among species of Axiidea. The elements of pattern *Ax2* that differ

318   from pattern *Ax1* support the node joining *Callianassa* and *Trypaea*.

319

320   *Evidence of significant codon usage bias in mitochondrial genomes at the infraorder level*

321

322   Figure 3 shows there is strong A+T bias in codon usage across the 33 shrimp

323   mitogenomes. Relative synonymous codon usage frequencies (RSCU) demonstrate distinct

324   preference for codons with A or T in the third codon position compared to other synonymous

325   codons. Counts of codons used and RSCU values can be found in Table S3. Among the 62

326   available codons, the four most used codons in all three infraorders are TTT (Phe), TTA (Leu),

327   ATT (Ile) and ATA (Met), all made up of solely A and T nucleotides. Even so, this preference

328   for A+T codons is stronger in Axiidea and Gebiidea mitogenomes and less so in Caridea, most

329   obvious for amino acids Asp (D), His (H), Asn (N) and Tyr (Y). Statistical comparisons show

330   that, for each amino acid, there are significant differences among the three infraorders in the

331   proportions of the codons being used (*p*-values in Fig. 3). A separate comparison for species with

332   pattern *Ax1* and *Ax2* within the infraorder Axiidea and also with those with the ground pattern

333   (*Gr*) do not reveal any substantial difference in their codon usage bias (Fig. S3). The MDS plot

334   shows that, for the most part, members of each infraorder cluster together and are largely distinct

335   from the samples from the other infraorders (Fig. 4). A sample of the Gebiidea, *T. kelanang*, is a

336   maverick, being placed well inside the Caridea cluster and remote from the other members of its

337   infraorder. It also has a very low AT content for this group, being more similar to caridean

338   shrimps. In this context, it is noteworthy that the species is placed as the most basal member of

339   the Gebiidea and is also the only member of the infraorder that has the primitive pancrustacean

340   gene order, which it shares with all other of the members of the Caridea with the exception of

341   some species with minor derived rearrangements (Fig. 2B).

342

343   **Discussion**

344   The five new decapod mitogenomes presented in this study considerably expand the

345   number of samples of Axiidea and Caridea currently available for mitogenome-based

346   phylogenetics and comparative mitogenomic studies. Members of the infraorder Gebiidea were

347   also included in this analysis due to its taxonomic history of having been placed with Axiidea in

348   the infraorder Thalassinidea, prior to obtaining recent recognition as its own separate infraorder

349   (Ahyong & O'Meally 2004; Crandall et al. 2000; Robles et al. 2009; Timm & Bracken-Grissom

350   2015). The mitogenomic features of these taxa are generally consistent with those for the

351   Decapoda and for the three infraorders (Kim et al. 2013; Lin et al. 2012; Miller et al. 2005)

352   (Table S1). In addition, the high AT content (58.9 – 73.6%) observed in all of the mitogenomes

353   utilized in this study is typical for the Crustacea and the Arthropoda (Cameron 2014; Cook et al.

354   2005; Lin et al. 2012; Shen et al. 2015).

355   It has been suggested that evolutionary rate and the frequency of rearrangements are

356   independent (Gissi et al. 2008) and this is consistent with results depicted in Fig. 2, which

357   indicate no obvious correlations between the size and distribution of rearrangements and branch

358   lengths (substitution rate) within and between groups. Despite this general observation some

359   studies have noted an association between higher substitution rates and the occurrence of

360   mitogenome rearrangements involving a transfer of genes between strands (e.g. Stokkan et al.

361   (2016). Although it is expected that codon usage varies between major groups of organisms and

362   among animal phyla (Castellana et al. 2011; Gissi et al. 2008; Prat et al. 2009), finding

363   substantial codon usage differences at the infraorder level is somewhat unusual. It is becoming

364   more apparent now that there are divergent patterns in AT content among orders in insects and

365   among the major taxonomic groups in the Malacostraca (see Table 1 in Sun et al. (2009) and

366   Table 1 in Shen et al. (2015)). Studies examining patterns of codon usage in mitogenomes have

367   failed to observe differences at finer taxonomic scales within insects and crustaceans (Cook et al.

368   2005; Shen et al. 2015; Sun et al. 2009).

369   Elevated mutational pressure is thought to be a major driver of non-random

370   mitochondrial synonymous variation. However, selection of optimal codons for translational

371 efficiency and genetic drift is also thought to play a role (Castellana et al. 2011; Jia & Higgs

372 2008; Prat et al. 2009). It is tempting to speculate that the distinctive pattern of codon usage in

373 the Axiidea, and the frequency and extent of mitogenome gene order rearrangements may be

374 correlated with the acquisition of specialized adaptations by members of this infraorder to largely

375 burrowing lifestyles (Lin et al. 2012; Sakai 2011) compared to the members of the Caridea,

376 which are mostly free living (Bracken et al. 2009).

377   The phylogenetic analyses using whole mitogenomes support the monophyly of each of

378 the three infraorders, Axiidea, Gebiidea and Caridea, with inclusion of more comprehensive

379 taxon sampling than previous studies (Lin et al. 2012; Shen et al. 2013; Shen et al. 2015; Tan et

380 al. 2015). Further, the members of Axiidea have a common gene rearrangement that is a potential

381 synapomorphy of the infraorder, and therefore also supports the monophyly of this group. A

382 notable exception to these results is the analysis of Shen et al. (2015), which supported a non-

383 monophyletic Gebiidea, by placing *T. kelanang* inside a lineage comprising Acheleta,

384 Polychelida and Caridea. Other studies using a combination of mitochondrial and nuclear gene

385 fragments (Bracken et al. 2009; Robles et al. 2009) also support the monophyly of these groups,

386 but their relationships with each other and other decapod infraorders have yet to be resolved (Tan

387 et al. 2015). A caveat of our findings and those of other studies is that monophyly cannot be fully

388 tested without comprehensive taxonomic and gene sampling and the inclusion of species from all

389 decapod infraorders (Timm & Bracken-Grissom 2015).

390   The contribution of mitogenomic data for more species will be particularly important for

391 exploring and testing evolutionary relationships within Axiidea, given its lack of broad

392 taxonomic representation on the current evolutionary tree. Two of the mitogenomes contributed

393 in this study (*C. ceramica*, *T. australiensis*) belong to the family Callianassidae, a diverse group

394 of axiidean shrimps adapted to digging in soft marine sediments. Over 100 species placed in 22

395 genera and divided between several subfamilies are recognized from this family (Sakai 2011).

396 However, relationships among the major groups and the definition of subfamily and generic level

397 boundaries are contentious within the Callianassidae (Poore et al. 2014; Sakai 2011). It is

398 noteworthy that for the Axiidea, the tree-based relationships and the mitogenome rearrangements

399 are entirely consistent with the infraorder, family, subfamily and genus level relationships for the

400 samples included (Fig. 2), and that the phylogenetic tree and the *Ax1* rearrangement pattern

401 further supports the monophyly of the Axiidea.

402   The potential for mitochondrial rearrangement to act as "super" characters for

403   phylogenetic estimation for the arthropod has been recognised by a number of authors (Boore et

404   al. 1998; Dowton & Austin 1999; Dowton et al. 2002). This study further supports this position

405   but also notes that the distribution of rearrangements is uneven across the tree generated in this

406   study and the larger analysis by Tan et al. (2015). Thus, while novel gene order attributes act as

407   useful phylogenetic characters for the Axiidea and Gebiidea, their usefulness appear to be limited

408   for the Caridea, even though the age of the lineages overlap. Similarly, for other major

409   crustaceans groups, lobsters (Astacidea and Achelata), crabs (Brachyura) and penaeid shrimps

410   (Dendrobranchiata), rearrangements are largely absent or minor (Shen et al. 2015; Tan et al.

411   2015), but freshwater crayfish (Astacidea) and anomuran crabs (Anomura) are phylogenetic

412   "hotspots" for mitogenome gene order evolution in both the number and scale of rearrangements.

413   As pointed out by Mao et al. (2014), far greater sampling is required to adequately test the

414   phylogenetic utility of observed mitogenome gene rearrangements and identification of suitable

415   models for investigating the evolutionary and molecular drivers that shape the organization and

416   architecture of animal mitogenomes.

417   In this regard, rapid and efficient methods for mitogenome sequencing using next-

418   generation sequencing (NGS) platforms will accelerate this task (Gan et al. 2014a; Timmermans

419   et al. 2016). Unlike more classical methods utilizing long-range PCR to amplify the mitogenome

420   into a smaller number of fragments with universal primers, followed by Sanger sequencing (Lin

421   et al. 2012; Miller et al. 2004; Shen et al. 2013), the primer-free and shotgun nature of NGS will

422   likely increase the discovery of mitogenome rearrangements as it makes no assumptions about

423   the pre-existing gene order of the species under study. In fact, the availability of a reference

424   whole mitogenome will improve primer design and consequently the success rate of complete

425   mitogenome recovery from members of the same genus (or family) using long-range PCR. It is

426   envisaged with the advent of third generation sequencing technology such as PacBio (Rhoads &

427   Au 2015) and Nanopore (Branton et al. 2008) sequencing, problematic and repetitive regions

428   commonly associated with the control region can be readily resolved and confirmed.

429   Lastly, while preparing the dataset for analysis, we identified several misannotated

430   mitogenomes on NCBI (Table 3) although these misannotations were absent in their related

431   publications i.e. the correct gene coordinates and orientations were reported by the authors of in

432   their respective publications. We postulate that these discrepancies may have arisen due to errors

433   during the submission of these mitogenomes to public databases[1]. It is also possible, though less

434   likely, that the mitogenomes may have been erroneously edited when they were reviewed by

435   NCBI staff. Hence, this highlights that although there is tremendous gain from having a growing

436   number of mitogenome submissions to public databases as molecular resources, the accuracy of

437   annotations should not be assumed and it is prudent to re-evaluate species with any form of gene

438   order rearrangements or related anomalies before inclusion in datasets for comparative analyses.

439

440   Acknowledgements

441   MHT and HMG would like to acknowledge the Monash University Malaysia High Performance

442   Computing infrastructure for the provision of computational resources. We are also grateful to C.

443   Tudge for collecting and providing the *Trypaea australiensis* sample and Joanne Taylor,

444   Museum Victoria, for making it available. The authors are also grateful for helpful comments of

445   the anonymous reviews that significantly improved the manuscript.

446

447

448   References

449   Abascal F, Zardoya R, and Telford MJ. 2010. TranslatorX: multiple alignment of nucleotide sequences
450   guided by amino acid translations. *Nucleic Acids Research* 38:W7-W13. 10.1093/nar/gkq291

451   Aberer AJ, Kobert K, and Stamatakis A. 2014. ExaBayes: Massively Parallel Bayesian Tree Inference for
452   the Whole-Genome Era. *Molecular biology and evolution* 31:2553-2556.
453   10.1093/molbev/msu236

454   Addinsoft. 2010. XLSTAT 2015, Data analysis and statistics software for Microsoft Excel. Addinsoft Paris,
455   France.

456   Ahyong ST, and O'Meally D. 2004. Phylogeny of the Decapoda Reptantia: resolution using three
457   molecular loci and morphology. *The Raffles Bulletin of Zoology* 52:673-693.

458   Alikhan N-F, Petty NK, Ben Zakour NL, and Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple
459   prokaryote genome comparisons. *BMC Genomics* 12:1-10. 10.1186/1471-2164-12-402

460   Bauzà-Ribot MM, Jaume D, Juan C, and Pons J. 2009. The complete mitochondrial genome of the
461   subterranean crustacean *Metacrangonyx longipes* (Amphipoda): A unique gene order and
462   extremely short control region. *Mitochondrial DNA* 20:88-99. 10.1080/19401730902964417

463   Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, and Stadler PF.
464   2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular
465   Phylogenetics and Evolution* 69:313-319. http://dx.doi.org/10.1016/j.ympev.2012.08.023

466   Boore JL. 2006. The use of genome-level characters for phylogenetic reconstruction. *Trends in Ecology &
467   Evolution* 21:439-446. http://dx.doi.org/10.1016/j.tree.2006.05.009

---

[1] At the time of writing, notifications have been submitted to NCBI to alert them of inconsistencies in the four mitogenomes reported in this study.

468   Boore JL, Lavrov DV, and Brown WM. 1998. Gene translocation links insects and crustaceans. *Nature*
469   392:667-668.
470   Bracken HD, De Grave S, and Felder DL. 2009. Phylogeny of the infraorder Caridea based on
471   mitochondrial and nuclear genes (Crustacea: Decapoda). *Decapod crustacean*
472   *phylogenetics*:281-305.
473   Branton D, Deamer DW, Marziali A, Bayley H, Benner SA, Butler T, Di Ventra M, Garaj S, Hibbs A, Huang
474   X, Jovanovich SB, Krstic PS, Lindsay S, Ling XS, Mastrangelo CH, Meller A, Oliver JS, Pershin YV,
475   Ramsey JM, Riehn R, Soni GV, Tabard-Cossa V, Wanunu M, Wiggin M, and Schloss JA. 2008. The
476   potential and challenges of nanopore sequencing. *Nat Biotech* 26:1146-1153.
477   Cameron SL. 2014. Insect Mitochondrial Genomics: Implications for Evolution and Phylogeny. *Annual*
478   *Review of Entomology* 59:95-117. doi:10.1146/annurev-ento-011613-162007
479   Capella-Gutiérrez S, Silla-Martínez JM, and Gabaldón T. 2009. trimAl: a tool for automated alignment
480   trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972-1973.
481   10.1093/bioinformatics/btp348
482   Castellana S, Vicario S, and Saccone C. 2011. Evolutionary Patterns of the Mitochondrial Genome in
483   Metazoa: Exploring the Role of Mutation and Selection in Mitochondrial Protein–Coding Genes.
484   *Genome Biology and Evolution* 3:1067-1079. 10.1093/gbe/evr040
485   Chong RA, and Mueller RL. 2013. Evolution Along the Mutation Gradient in the Dynamic Mitochondrial
486   Genome of Salamanders. *Genome Biology and Evolution* 5:1652-1660. 10.1093/gbe/evt119
487   Cook CE, Yue Q, and Akam M. 2005. Mitochondrial genomes suggest that hexapods and crustaceans are
488   mutually paraphyletic. *Proceedings of the Royal Society of London B: Biological Sciences*
489   272:1295-1304. 10.1098/rspb.2004.3042
490   Crandal KA, Harris DJ, and Fetzner JW. 2000. The monophyletic origin of freshwater crayfish estimated
491   from nuclear and mitochondrial DNA sequences. *Proceedings of the Royal Society of London B:*
492   *Biological Sciences* 267:1679-1686. 10.1098/rspb.2000.1195
493   Darriba D, Taboada GL, Doallo R, and Posada D. 2012. jModelTest 2: more models, new heuristics and
494   parallel computing. *Nat Meth* 9:772-772.
495   http://www.nature.com/nmeth/journal/v9/n8/abs/nmeth.2109.html#supplementary-
496   information
497   Dowton M, and Austin AD. 1999. Evolutionary dynamics of a mitochondrial rearrangement "hot spot" in
498   the Hymenoptera. *Molecular biology and evolution* 16:298-309.
499   Dowton M, Castro LR, and Austin AD. 2002. Mitochondrial gene rearrangements as phylogenetic
500   characters in the invertebrates: the examination of genome 'morphology'. *Invertebrate*
501   *Systematics* 16:345-356. http://dx.doi.org/10.1071/IS02003
502   Dowton M, Castro RL, Campbell LS, Bargon DS, and Austin DA. 2003. Frequent Mitochondrial Gene
503   Rearrangements at the Hymenopteran nad3–nad5 Junction. *Journal of Molecular Evolution*
504   56:517-526. 10.1007/s00239-002-2420-3
505   Fahrein K, Talarico G, Braband A, and Podsiadlowski L. 2007. The complete mitochondrial genome of
506   *Pseudocellus pearsei* (Chelicerata: Ricinulei) and a comparison of mitochondrial gene
507   rearrangements in Arachnida. *BMC Genomics* 8:1-14. 10.1186/1471-2164-8-386
508   Felder DL, and Robles R. 2009. Molecular phylogeny of the family Callianassidae based on preliminary
509   analyses of two mitochondrial genes. *Decapod crustacean phylogenetics Crustacean issues*
510   18:327-342.
511   Gan HM, Schultz MB, and Austin CM. 2014a. Integrated shotgun sequencing and bioinformatics pipeline
512   allows ultra-fast mitogenome recovery and confirms substantial gene rearrangements in
513   Australian freshwater crayfishes. *BMC Evol Biol* 14:19. 10.1186/1471-2148-14-19
514   1471-2148-14-19 [pii]

515   Gan HY, Gan HM, Lee YP, and Austin CM. 2014b. The complete mitochondrial genome of the bass yabby
516   *Trypaea australiensis* Dana 1852, (Crustacea; Decapoda; Callianassidae) – a new gene order for
517   the Decapoda. *Mitochondrial DNA*:1-2. 10.3109/19401736.2014.989516
518   Gan HY, Gan HM, Tan MH, Lee YP, and Austin CM. 2015. The complete mitogenome of the hermit crab
519   *Clibanarius infraspinatus* (Hilgendorf, 1869), (Crustacea; Decapoda; Diogenidae) – a new gene
520   order for the Decapoda. *Mitochondrial DNA*:1-2. 10.3109/19401736.2014.1003862
521   García-Machado E, Pempera M, Dennebouy N, Oliva-Suarez M, Mounolou J-C, and Monnerot M. 1999.
522   Mitochondrial Genes Collectively Suggest the Paraphyly of Crustacea with Respect to Insecta.
523   *Journal of Molecular Evolution* 49:142-149. 10.1007/pl00006527
524   Gissi C, Iannelli F, and Pesole G. 2004. Complete mtDNA of *Ciona intestinalis* Reveals Extensive Gene
525   Rearrangement and the Presence of an atp8 and an Extra trnM Gene in Ascidians. *Journal of*
526   *Molecular Evolution* 58:376-389. 10.1007/s00239-003-2559-6
527   Gissi C, Iannelli F, and Pesole G. 2008. Evolution of the mitochondrial genome of Metazoa as exemplified
528   by comparison of congeneric species. *Heredity* 101:301-320.
529   http://www.nature.com/hdy/journal/v101/n4/suppinfo/hdy200862s1.html
530   Jia W, and Higgs PG. 2008. Codon Usage in Mitochondrial Genomes: Distinguishing Context-Dependent
531   Mutation from Translational Selection. *Molecular biology and evolution* 25:339-351.
532   10.1093/molbev/msm259
533   Jiang ZJ, Castoe TA, Austin CC, Burbrink FT, Herron MD, McGuire JA, Parkinson CL, and Pollock DD. 2007.
534   Comparative mitochondrial genomics of snakes: extraordinary substitution rate dynamics and
535   functionality of the duplicate control region. *BMC Evolutionary Biology* 7:1-14. 10.1186/1471-
536   2148-7-123
537   Katoh K, and Standley DM. 2013. MAFFT multiple sequence alignment software version 7:
538   improvements in performance and usability. *Molecular biology and evolution* 30:772-780.
539   Kilpert F, and Podsiadlowski L. 2006. The complete mitochondrial genome of the common sea slater,
540   *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region
541   features. *BMC Genomics* 7:1-18. 10.1186/1471-2164-7-241
542   Kim S, Ahn D-H, Park J-K, Kim S-J, Choi H-G, and Min G-S. 2013. The complete mitochondrial genome of
543   the Japanese ghost shrimp *Nihonotrypaea japonica* (Crustacea, Decapoda, Axiidea).
544   *Mitochondrial DNA* 24:243-245. 10.3109/19401736.2012.752480
545   Kim S, Park M-H, Jung J-H, Ahn D-H, Sultana T, Kim S, Park J-K, Choi H-G, and Min G-S. 2012. The
546   mitochondrial genomes of Cambaroides similis and Procambarus clarkii (Decapoda: Astacidea:
547   Cambaridae): the phylogenetic implications for Reptantia. *Zoologica Scripta* 41:281-292.
548   10.1111/j.1463-6409.2012.00534.x
549   Li X, Huang Y, and Lei F. 2015. Comparative mitochondrial genomics and phylogenetic relationships of
550   the *Crossoptilon* species (Phasianidae, Galliformes). *BMC Genomics* 16:1-12. 10.1186/s12864-
551   015-1234-9
552   Lin F-J, Liu Y, Sha Z, Tsang L, Chu K, Chan T-Y, Liu R, and Cui Z. 2012. Evolution and phylogeny of the mud
553   shrimps (Crustacea: Decapoda) revealed from complete mitochondrial genomes. *BMC Genomics*
554   13:631.
555   Mao M, Gibson T, and Dowton M. 2014. Evolutionary Dynamics of the Mitochondrial Genome in the
556   Evaniomorpha (Hymenoptera)—A Group with an Intermediate Rate of Gene Rearrangement.
557   *Genome Biology and Evolution* 6:1862-1874. 10.1093/gbe/evu145
558   Miller AD, Murphy NP, Burridge CP, and Austin CM. 2005. Complete Mitochondrial DNA Sequences of
559   the Decapod Crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii*
560   (Palaemonidae). *Marine Biotechnology* 7:339-349. 10.1007/s10126-004-4077-8

561 Miller AD, Nguyen TTT, Burridge CP, and Austin CM. 2004. Complete mitochondrial DNA sequence of the
562   Australian freshwater crayfish, *Cherax destructor* (Crustacea: Decapoda: Parastacidae): a novel
563   gene order revealed. *Gene* 331:65-72. http://dx.doi.org/10.1016/j.gene.2004.01.022
564 Murphy NP, and Austin CM. 2004. Multiple origins of the endemic Australian *Macrobrachium* (Decapoda :
565   Palaemonidae) based on 16S rRNA mitochondrial sequences. *Australian Journal of Zoology*
566   52:549-559. http://dx.doi.org/10.1071/ZO03062
567 Oliveira DCSG, Raychoudhury R, Lavrov DV, and Werren JH. 2008. Rapidly Evolving Mitochondrial
568   Genome and Directional Selection in Mitochondrial Genes in the Parasitic Wasp *Nasonia*
569   (Hymenoptera: Pteromalidae). *Molecular biology and evolution* 25:2167-2180.
570   10.1093/molbev/msn159
571 Page TJ, von Rintelen K, and Hughes JM. 2007. An island in the stream: Australia's place in the
572   cosmopolitan world of Indo-West Pacific freshwater shrimp (Decapoda: Atyidae: Caridina).
573   *Molecular Phylogenetics and Evolution* 43:645-659.
574   http://dx.doi.org/10.1016/j.ympev.2006.08.007
575 Peng Y, Leung HCM, Yiu SM, and Chin FYL. 2012. IDBA-UD: a de novo assembler for single-cell and
576   metagenomic sequencing data with highly uneven depth. *Bioinformatics* 28:1420-1428.
577   10.1093/bioinformatics/bts174
578 Poore GC. 1994. A phylogeny of the families of Thalassinidea (Crustacea: Decapoda) with keys to families
579   and genera. *Memoirs of the Museum of Victoria* 54:79-120.
580 Poore GCB, Ahyong ST, Bracken-Grissom HD, Chan T-Y, Chu KH, Crandall KA, Dworschak PC, Felder DL,
581   Feldmann RM, Hyžný M, Karasawa H, Lemaitre R, Komai T, Li X, Mantelatto FL, Martin JW, Ngoc-
582   Ho N, Robles R, Schweitzer CE, Tamaki A, Tsang LM, and Tudge CC. 2014. On stabilising the
583   names of the infraorders of thalassinidean shrimps, Axiidea de Saint Laurent, 1979 and Gebiidea
584   de Saint Laurent, 1979 (Decapoda). *Crustaceana* 87:1258-1272.
585   doi:http://dx.doi.org/10.1163/15685403-00003354
586 Poulsen JY, Byrkjedal I, Willassen E, Rees D, Takeshima H, Satoh TP, Shinohara G, Nishida M, and Miya M.
587   2013. Mitogenomic sequences and evidence from unique gene rearrangements corroborate
588   evolutionary relationships of myctophiformes (Neoteleostei). *BMC Evolutionary Biology* 13:1-22.
589   10.1186/1471-2148-13-111
590 Prat Y, Fromer M, Linial N, and Linial M. 2009. Codon usage is associated with the evolutionary age of
591   genes in metazoan genomes. *BMC Evolutionary Biology* 9:1-12. 10.1186/1471-2148-9-285
592 Qian G, Zhao Q, Wang AN, Zhu LIN, Zhou K, and Sun H. 2011. Two new decapod (Crustacea,
593   Malacostraca) complete mitochondrial genomes: bearings on the phylogenetic relationships
594   within the Decapoda. *Zoological Journal of the Linnean Society* 162:471-481. 10.1111/j.1096-
595   3642.2010.00686.x
596 Rhoads A, and Au KF. 2015. PacBio Sequencing and Its Applications. *Genomics, Proteomics &*
597   *Bioinformatics* 13:278-289. http://dx.doi.org/10.1016/j.gpb.2015.08.002
598 Rice P, Longden I, and Bleasby A. 2000. EMBOSS: The European Molecular Biology Open Software Suite.
599   *Trends in Genetics* 16:276-277. 10.1016/s0168-9525(00)02024-2
600 Robles R, Tudge CC, Dworschak PC, Poore GC, and Felder D. 2009. Molecular phylogeny of the
601   Thalassinidea based on nuclear and mitochondrial genes. *Decapod crustacean*
602   *phylogenetics*:309-326.
603 Rota-Stabelli O, Lartillot N, Philippe H, and Pisani D. 2013. Serine Codon-Usage Bias in Deep
604   Phylogenomics: Pancrustacean Relationships as a Case Study. *Systematic Biology* 62:121-133.
605   10.1093/sysbio/sys077
606 Sakai K. 2011. *Axioidea of the world and a reconsideration of the Callianassoidea (Decapoda,*
607   *Thalassinidea, Callianassida)*: Brill.

608   Shao R, Barker SC, Mitani H, Aoki Y, and Fukunaga M. 2005. Evolution of Duplicate Control Regions in the
609   Mitochondrial Genomes of Metazoa: A Case Study with Australasian Ixodes Ticks. *Molecular*
610   *biology and evolution* 22:620-629. 10.1093/molbev/msi047
611   Sharp PM, and Li W-H. 1987. The codon adaptation index-a measure of directional synonymous codon
612   usage bias, and its potential applications. *Nucleic Acids Research* 15:1281-1295.
613   10.1093/nar/15.3.1281
614   Shen H, Braband A, and Scholtz G. 2013. Mitogenomic analysis of decapod crustacean phylogeny
615   corroborates traditional views on their relationships. *Molecular Phylogenetics and Evolution*
616   66:776-789. http://dx.doi.org/10.1016/j.ympev.2012.11.002
617   Shen X, Tian M, Yan B, and Chu K. 2015. Phylomitogenomics of Malacostraca (Arthropoda: Crustacea).
618   *Acta Oceanologica Sinica* 34:84-92. 10.1007/s13131-015-0583-1
619   Shoemaker DD, Dyer KA, Ahrens M, McAbee K, and Jaenike J. 2004. Decreased diversity but increased
620   substitution rate in host mtDNA as a consequence of Wolbachia endosymbiont infection.
621   *Genetics* 168:2049-2058.
622   Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large
623   phylogenies. *Bioinformatics*. 10.1093/bioinformatics/btu033
624   Sun Z, Wan D-G, Murphy RW, Ma L, Zhang X-S, and Huang D-W. 2009. Comparison of base composition
625   and codon usage in insect mitochondrial genomes. *Genes & Genomics* 31:65-71.
626   10.1007/bf03191139
627   Tan MH, Gan HM, Schultz MB, and Austin CM. 2015. MitoPhAST, a new automated mitogenomic
628   phylogeny tool in the post-genomic era with a case study of 89 decapod mitogenomes including
629   eight new freshwater crayfish mitogenomes. *Molecular Phylogenetics and Evolution* 85:180-188.
630   http://dx.doi.org/10.1016/j.ympev.2015.02.009
631   Timm L, and Bracken-Grissom HD. 2015. The forest for the trees: evaluating molecular phylogenies with
632   an emphasis on higher-level Decapoda. *JOURNAL OF CRUSTACEAN BIOLOGY* 35:577-592.
633   doi:http://dx.doi.org/10.1163/1937240X-00002371
634   Timmermans MJTN, Viberg C, Martin G, Hopkins K, and Vogler AP. 2016. Rapid assembly of
635   taxonomically validated mitochondrial genomes from historical insect collections. *Biological*
636   *Journal of the Linnean Society* 117:83-95. 10.1111/bij.12552
637   Whittle CA, and Extavour CG. 2015. Codon and Amino Acid Usage Are Shaped by Selection Across
638   Divergent Model Organisms of the Pancrustacea. *G3: Genes|Genomes|Genetics* 5:2307-2321.
639   10.1534/g3.115.021402
640
641   Abascal F, Zardoya R, and Telford MJ. 2010. TranslatorX: multiple alignment of nucleotide sequences
642   guided by amino acid translations. *Nucleic Acids Research* 38:W7-W13. 10.1093/nar/gkq291
643   Aberer AJ, Kobert K, and Stamatakis A. 2014. ExaBayes: Massively Parallel Bayesian Tree Inference for
644   the Whole-Genome Era. *Molecular biology and evolution* 31:2553-2556.
645   10.1093/molbev/msu236
646   Addinsoft. 2010. XLSTAT 2015, Data analysis and statistics software for Microsoft Excel. Addinsoft Paris,
647   France.
648   Ahyong ST, and O'Meally D. 2004. Phylogeny of the Decapoda Reptantia: resolution using three
649   molecular loci and morphology. *The Raffles Bulletin of Zoology* 52:673-693.
650   Alikhan N-F, Petty NK, Ben Zakour NL, and Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple
651   prokaryote genome comparisons. *BMC Genomics* 12:1-10. 10.1186/1471-2164-12-402
652   Bauzà-Ribot MM, Jaume D, Juan C, and Pons J. 2009. The complete mitochondrial genome of the
653   subterranean crustacean *Metacrangonyx longipes* (Amphipoda): A unique gene order and
654   extremely short control region. *Mitochondrial DNA* 20:88-99. 10.1080/19401730902964417

655 Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, and Stadler PF.
656   2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular*
657   *Phylogenetics and Evolution* 69:313-319. http://dx.doi.org/10.1016/j.ympev.2012.08.023
658 Boore JL. 2006. The use of genome-level characters for phylogenetic reconstruction. *Trends in Ecology &*
659   *Evolution* 21:439-446. http://dx.doi.org/10.1016/j.tree.2006.05.009
660 Boore JL, Lavrov DV, and Brown WM. 1998. Gene translocation links insects and crustaceans. *Nature*
661   392:667-668.
662 Bracken HD, De Grave S, and Felder DL. 2009. Phylogeny of the infraorder Caridea based on
663   mitochondrial and nuclear genes (Crustacea: Decapoda). *Decapod crustacean*
664   *phylogenetics*:281-305.
665 Branton D, Deamer DW, Marziali A, Bayley H, Benner SA, Butler T, Di Ventra M, Garaj S, Hibbs A, Huang
666   X, Jovanovich SB, Krstic PS, Lindsay S, Ling XS, Mastrangelo CH, Meller A, Oliver JS, Pershin YV,
667   Ramsey JM, Riehn R, Soni GV, Tabard-Cossa V, Wanunu M, Wiggin M, and Schloss JA. 2008. The
668   potential and challenges of nanopore sequencing. *Nat Biotech* 26:1146-1153.
669 Cameron SL. 2014. Insect Mitochondrial Genomics: Implications for Evolution and Phylogeny. *Annual*
670   *Review of Entomology* 59:95-117. doi:10.1146/annurev-ento-011613-162007
671 Capella-Gutiérrez S, Silla-Martínez JM, and Gabaldón T. 2009. trimAl: a tool for automated alignment
672   trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972-1973.
673   10.1093/bioinformatics/btp348
674 Castellana S, Vicario S, and Saccone C. 2011. Evolutionary Patterns of the Mitochondrial Genome in
675   Metazoa: Exploring the Role of Mutation and Selection in Mitochondrial Protein–Coding Genes.
676   *Genome Biology and Evolution* 3:1067-1079. 10.1093/gbe/evr040
677 Castresana J. 2000. Selection of Conserved Blocks from Multiple Alignments for Their Use in
678   Phylogenetic Analysis. *Molecular biology and evolution* 17:540-552.
679 Chong RA, and Mueller RL. 2013. Evolution Along the Mutation Gradient in the Dynamic Mitochondrial
680   Genome of Salamanders. *Genome Biology and Evolution* 5:1652-1660. 10.1093/gbe/evt119
681 Cook CE, Yue Q, and Akam M. 2005. Mitochondrial genomes suggest that hexapods and crustaceans are
682   mutually paraphyletic. *Proceedings of the Royal Society of London B: Biological Sciences*
683   272:1295-1304. 10.1098/rspb.2004.3042
684 Crandall KA, Harris DJ, and Fetzner JW. 2000. The monophyletic origin of freshwater crayfish estimated
685   from nuclear and mitochondrial DNA sequences. *Proceedings of the Royal Society of London B:*
686   *Biological Sciences* 267:1679-1686. 10.1098/rspb.2000.1195
687 Dowton M, and Austin AD. 1999. Evolutionary dynamics of a mitochondrial rearrangement "hot spot" in
688   the Hymenoptera. *Molecular biology and evolution* 16:298-309.
689 Dowton M, Castro LR, and Austin AD. 2002. Mitochondrial gene rearrangements as phylogenetic
690   characters in the invertebrates: the examination of genome 'morphology'. *Invertebrate*
691   *Systematics* 16:345-356. http://dx.doi.org/10.1071/IS02003
692 Dowton M, Castro RL, Campbell LS, Bargon DS, and Austin DA. 2003. Frequent Mitochondrial Gene
693   Rearrangements at the Hymenopteran nad3–nad5 Junction. *Journal of Molecular Evolution*
694   56:517-526. 10.1007/s00239-002-2420-3
695 Fahrein K, Talarico G, Braband A, and Podsiadlowski L. 2007. The complete mitochondrial genome of
696   *Pseudocellus pearsei* (Chelicerata: Ricinulei) and a comparison of mitochondrial gene
697   rearrangements in Arachnida. *BMC Genomics* 8:1-14. 10.1186/1471-2164-8-386
698 Felder DL, and Robles R. 2009. Molecular phylogeny of the family Callianassidae based on preliminary
699   analyses of two mitochondrial genes. *Decapod crustacean phylogenetics Crustacean issues*
700   18:327-342.

701 Gan HM, Schultz MB, and Austin CM. 2014a. Integrated shotgun sequencing and bioinformatics pipeline
702   allows ultra-fast mitogenome recovery and confirms substantial gene rearrangements in
703   Australian freshwater crayfishes. *BMC Evol Biol* 14:19. 10.1186/1471-2148-14-19
704 1471-2148-14-19 [pii]
705 Gan HY, Gan HM, Lee YP, and Austin CM. 2014b. The complete mitochondrial genome of the bass yabby
706   *Trypaea australiensis* Dana 1852, (Crustacea; Decapoda; Callianassidae) – a new gene order for
707   the Decapoda. *Mitochondrial DNA*:1-2. 10.3109/19401736.2014.989516
708 Gan HY, Gan HM, Tan MH, Lee YP, and Austin CM. 2015. The complete mitogenome of the hermit crab
709   *Clibanarius infraspinatus* (Hilgendorf, 1869), (Crustacea; Decapoda; Diogenidae) – a new gene
710   order for the Decapoda. *Mitochondrial DNA*:1-2. 10.3109/19401736.2014.1003862
711 García-Machado E, Pempera M, Dennebouy N, Oliva-Suarez M, Mounolou J-C, and Monnerot M. 1999.
712   Mitochondrial Genes Collectively Suggest the Paraphyly of Crustacea with Respect to Insecta.
713   *Journal of Molecular Evolution* 49:142-149. 10.1007/pl00006527
714 Gissi C, Iannelli F, and Pesole G. 2004. Complete mtDNA of *Ciona intestinalis* Reveals Extensive Gene
715   Rearrangement and the Presence of an atp8 and an Extra trnM Gene in Ascidians. *Journal of
716   Molecular Evolution* 58:376-389. 10.1007/s00239-003-2559-6
717 Gissi C, Iannelli F, and Pesole G. 2008. Evolution of the mitochondrial genome of Metazoa as exemplified
718   by comparison of congeneric species. *Heredity* 101:301-320.
719   http://www.nature.com/hdy/journal/v101/n4/suppinfo/hdy200862s1.html
720 Jia W, and Higgs PG. 2008. Codon Usage in Mitochondrial Genomes: Distinguishing Context-Dependent
721   Mutation from Translational Selection. *Molecular biology and evolution* 25:339-351.
722   10.1093/molbev/msm259
723 Jiang ZJ, Castoe TA, Austin CC, Burbrink FT, Herron MD, McGuire JA, Parkinson CL, and Pollock DD. 2007.
724   Comparative mitochondrial genomics of snakes: extraordinary substitution rate dynamics and
725   functionality of the duplicate control region. *BMC Evolutionary Biology* 7:1-14. 10.1186/1471-
726   2148-7-123
727 Katoh K, and Standley DM. 2013. MAFFT multiple sequence alignment software version 7:
728   improvements in performance and usability. *Molecular biology and evolution* 30:772-780.
729 Kilpert F, and Podsiadlowski L. 2006. The complete mitochondrial genome of the common sea slater,
730   *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region
731   features. *BMC Genomics* 7:1-18. 10.1186/1471-2164-7-241
732 Kim S, Ahn D-H, Park J-K, Kim S-J, Choi H-G, and Min G-S. 2013. The complete mitochondrial genome of
733   the Japanese ghost shrimp *Nihonotrypaea japonica* (Crustacea, Decapoda, Axiidea).
734   *Mitochondrial DNA* 24:243-245. 10.3109/19401736.2012.752480
735 Kim S, Park M-H, Jung J-H, Ahn D-H, Sultana T, Kim S, Park J-K, Choi H-G, and Min G-S. 2012. The
736   mitochondrial genomes of Cambaroides similis and Procambarus clarkii (Decapoda: Astacidea:
737   Cambaridae): the phylogenetic implications for Reptantia. *Zoologica Scripta* 41:281-292.
738   10.1111/j.1463-6409.2012.00534.x
739 Li X, Huang Y, and Lei F. 2015. Comparative mitochondrial genomics and phylogenetic relationships of
740   the *Crossoptilon* species (Phasianidae, Galliformes). *BMC Genomics* 16:1-12. 10.1186/s12864-
741   015-1234-9
742 Lin F-J, Liu Y, Sha Z, Tsang L, Chu K, Chan T-Y, Liu R, and Cui Z. 2012. Evolution and phylogeny of the mud
743   shrimps (Crustacea: Decapoda) revealed from complete mitochondrial genomes. *BMC Genomics*
744   13:631.
745 Mao M, Gibson T, and Dowton M. 2014. Evolutionary Dynamics of the Mitochondrial Genome in the
746   Evaniomorpha (Hymenoptera)—A Group with an Intermediate Rate of Gene Rearrangement.
747   *Genome Biology and Evolution* 6:1862-1874. 10.1093/gbe/evu145

748   Miller AD, Murphy NP, Burridge CP, and Austin CM. 2005. Complete Mitochondrial DNA Sequences of
749   the Decapod Crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii*
750   (Palaemonidae). *Marine Biotechnology* 7:339-349. 10.1007/s10126-004-4077-8
751   Miller AD, Nguyen TTT, Burridge CP, and Austin CM. 2004. Complete mitochondrial DNA sequence of the
752   Australian freshwater crayfish, *Cherax destructor* (Crustacea: Decapoda: Parastacidae): a novel
753   gene order revealed. *Gene* 331:65-72. http://dx.doi.org/10.1016/j.gene.2004.01.022
754   Minh BQ, Nguyen MAT, and von Haeseler A. 2013. Ultrafast Approximation for Phylogenetic Bootstrap.
755   *Molecular biology and evolution*. 10.1093/molbev/mst024
756   Muhire BM, Varsani A, and Martin DP. 2014. SDT: A Virus Classification Tool Based on Pairwise Sequence
757   Alignment and Identity Calculation. *PLoS One* 9:e108277. 10.1371/journal.pone.0108277
758   Nguyen L-T, Schmidt HA, von Haeseler A, and Minh BQ. 2015. IQ-TREE: A Fast and Effective Stochastic
759   Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular biology and evolution*
760   32:268-274. 10.1093/molbev/msu300
761   Oliveira DCSG, Raychoudhury R, Lavrov DV, and Werren JH. 2008. Rapidly Evolving Mitochondrial
762   Genome and Directional Selection in Mitochondrial Genes in the Parasitic Wasp *Nasonia*
763   (Hymenoptera: Pteromalidae). *Molecular biology and evolution* 25:2167-2180.
764   10.1093/molbev/msn159
765   Peng Y, Leung HCM, Yiu SM, and Chin FYL. 2012. IDBA-UD: a de novo assembler for single-cell and
766   metagenomic sequencing data with highly uneven depth. *Bioinformatics* 28:1420-1428.
767   10.1093/bioinformatics/bts174
768   Poore GCB, Ahyong ST, Bracken-Grissom HD, Chan T-Y, Chu KH, Crandall KA, Dworschak PC, Felder DL,
769   Feldmann RM, Hyžný M, Karasawa H, Lemaitre R, Komai T, Li X, Mantelatto FL, Martin JW, Ngoc-
770   Ho N, Robles R, Schweitzer CE, Tamaki A, Tsang LM, and Tudge CC. 2014. On stabilising the
771   names of the infraorders of thalassinidean shrimps, Axiidea de Saint Laurent, 1979 and Gebiidea
772   de Saint Laurent, 1979 (Decapoda). *Crustaceana* 87:1258-1272.
773   doi:http://dx.doi.org/10.1163/15685403-00003354
774   Poulsen JY, Byrkjedal I, Willassen E, Rees D, Takeshima H, Satoh TP, Shinohara G, Nishida M, and Miya M.
775   2013. Mitogenomic sequences and evidence from unique gene rearrangements corroborate
776   evolutionary relationships of myctophiformes (Neoteleostei). *BMC Evolutionary Biology* 13:1-22.
777   10.1186/1471-2148-13-111
778   Prat Y, Fromer M, Linial N, and Linial M. 2009. Codon usage is associated with the evolutionary age of
779   genes in metazoan genomes. *BMC Evolutionary Biology* 9:1-12. 10.1186/1471-2148-9-285
780   Qian G, Zhao Q, Wang AN, Zhu LIN, Zhou K, and Sun H. 2011. Two new decapod (Crustacea,
781   Malacostraca) complete mitochondrial genomes: bearings on the phylogenetic relationships
782   within the Decapoda. *Zoological Journal of the Linnean Society* 162:471-481. 10.1111/j.1096-
783   3642.2010.00686.x
784   Rhoads A, and Au KF. 2015. PacBio Sequencing and Its Applications. *Genomics, Proteomics &*
785   *Bioinformatics* 13:278-289. http://dx.doi.org/10.1016/j.gpb.2015.08.002
786   Rice P, Longden I, and Bleasby A. 2000. EMBOSS: The European Molecular Biology Open Software Suite.
787   *Trends in Genetics* 16:276-277. 10.1016/s0168-9525(00)02024-2
788   Robles R, Tudge CC, Dworschak PC, Poore GC, and Felder D. 2009. Molecular phylogeny of the
789   Thalassinidea based on nuclear and mitochondrial genes. *Decapod crustacean*
790   *phylogenetics*:309-326.
791   Rota-Stabelli O, Lartillot N, Philippe H, and Pisani D. 2013. Serine Codon-Usage Bias in Deep
792   Phylogenomics: Pancrustacean Relationships as a Case Study. *Systematic Biology* 62:121-133.
793   10.1093/sysbio/sys077
794   Sakai K. 2011. *Axioidea of the world and a reconsideration of the Callianassoidea (Decapoda,*
795   *Thalassinidea, Callianassida)*: Brill.

796 Shao R, Barker SC, Mitani H, Aoki Y, and Fukunaga M. 2005. Evolution of Duplicate Control Regions in the
797   Mitochondrial Genomes of Metazoa: A Case Study with Australasian Ixodes Ticks. *Molecular*
798   *biology and evolution* 22:620-629. 10.1093/molbev/msi047
799 Sharp PM, and Li W-H. 1987. The codon adaptation index-a measure of directional synonymous codon
800   usage bias, and its potential applications. *Nucleic Acids Research* 15:1281-1295.
801   10.1093/nar/15.3.1281
802 Shen H, Braband A, and Scholtz G. 2013. Mitogenomic analysis of decapod crustacean phylogeny
803   corroborates traditional views on their relationships. *Molecular Phylogenetics and Evolution*
804   66:776-789. http://dx.doi.org/10.1016/j.ympev.2012.11.002
805 Shen X, Tian M, Yan B, and Chu K. 2015. Phylomitogenomics of Malacostraca (Arthropoda: Crustacea).
806   *Acta Oceanologica Sinica* 34:84-92. 10.1007/s13131-015-0583-1
807 Shoemaker DD, Dyer KA, Ahrens M, McAbee K, and Jaenike J. 2004. Decreased diversity but increased
808   substitution rate in host mtDNA as a consequence of Wolbachia endosymbiont infection.
809   *Genetics* 168:2049-2058.
810 Stokkan M, Jurado-Rivera JA, Juan C, Jaume D, and Pons J. 2016. Mitochondrial genome rearrangements
811   at low taxonomic levels: three distinct mitogenome gene orders in the genus Pseudoniphargus
812   (Crustacea: Amphipoda). *Mitochondrial DNA Part A* 27:3579-3589.
813   10.3109/19401736.2015.1079821
814 Sun Z, Wan D-G, Murphy RW, Ma L, Zhang X-S, and Huang D-W. 2009. Comparison of base composition
815   and codon usage in insect mitochondrial genomes. *Genes & Genomics* 31:65-71.
816   10.1007/bf03191139
817 Tan MH, Gan HM, Schultz MB, and Austin CM. 2015. MitoPhAST, a new automated mitogenomic
818   phylogeny tool in the post-genomic era with a case study of 89 decapod mitogenomes including
819   eight new freshwater crayfish mitogenomes. *Molecular Phylogenetics and Evolution* 85:180-188.
820   http://dx.doi.org/10.1016/j.ympev.2015.02.009
821 Timm L, and Bracken-Grissom HD. 2015. The forest for the trees: evaluating molecular phylogenies with
822   an emphasis on higher-level Decapoda. *JOURNAL OF CRUSTACEAN BIOLOGY* 35:577-592.
823   doi:http://dx.doi.org/10.1163/1937240X-00002371
824 Timmermans MJTN, Viberg C, Martin G, Hopkins K, and Vogler AP. 2016. Rapid assembly of
825   taxonomically validated mitochondrial genomes from historical insect collections. *Biological*
826   *Journal of the Linnean Society* 117:83-95. 10.1111/bij.12552
827 Whittle CA, and Extavour CG. 2015. Codon and Amino Acid Usage Are Shaped by Selection Across
828   Divergent Model Organisms of the Pancrustacea. *G3: Genes|Genomes|Genetics* 5:2307-2321.
829   10.1534/g3.115.021402
830
831

List of source and accession number of mitogenome samples.

	Callianassa ceramica	*Callianassa ceramica*	*Trypaea australiensis*	*Alpheus lobidens*	*Caridina* cf. *nilotica*	*Macrobrachium bullatum*
Family	Callianassidae	Callianassidae	Callianassidae	Alpheidae	Atyidae	Palaemonidae
Subfamily	Callianassinae	Callianassinae	Callianassinae	N/A	N/A	Palaemoninae
Location	Anglesea, South west of Geelong, Victoria	South of Port Authority Pier, Queenscliff, Victoria	Stony Point, Western Port Bay, Victoria	Nightcliff, Darwin, Northern Territory	Amy Ward Drive, Darwin, Northern Territory	Rapid Creek, Darwin, Northern Territory
Voucher	N/A	NMV J40715	NMV J40711	MAGNT Cr.18581	N/A	N/A
Verification	99.8% (660 bp *COI*)[1]	N/A	N/A	N/A	100% (326bp 16S)[2]	100% (447bp 16S)[3]
Accession #	KU362925.1	KU350630.1	KM501040.2	KP276147.1	KU726823.1	KM978918.1

[1] NMV J70519, Point Roadknight, Anglesea, Victoria

[2] Page et al. (2007) – DQ478508.1 – MAGNT Cr. 9399

[3] Murphy & Austin (2004) – AY282778.1

NMV = Museum Victoria; MAGNT = Museum and Art Galleries of the Northern Territory

List of samples and their corresponding accession numbers included in phylogenetic and comparative analyses.

Infraorder	Family	Species	Accession	Reference
Axiidea	**Callianassidae**	*Callianassa ceramica*	KU350630.1	**This study**
		Callianassa ceramica	KU362925.1	Gan et al. (2014b)
		Corallianassa coutierei	NC_020025.1	Shen et al. (2013)
		Nihonotrypaea japonica	NC_020351.1	Kim et al. (2013)
		Nihonotrypaea thermophile	NC_019610.1	Lin et al. (2012)
		Paraglypturus tonganus[1]	NC_024651.1	Kim et al. (2016b)
		Trypaea australiensis[2]	KM501040.2	**This study**
	Strahlaxiidae	*Neaxius glyptocercus*	NC_019609.1	Lin et al. (2012)
Gebiidea	**Thalassinidae**	*Thalassina kelanang*	NC_019608.1	Lin et al. (2012)
	Upogebiidae	*Austinogebia edulis*	NC_019606.1	Lin et al. (2012)
		Upogebia major	NC_019607.1	Lin et al. (2012)
		Upogebia pusilla	NC_020023.1	Shen et al. (2013)
		Upogebia yokoyai	NC_025943.1	Yang et al. (2016)
Caridea	**Alvinocarididae**	*Alvinocaris chelys*	NC_018778.1	Yang et al. (2012)
		Alvinocaris longirostris	NC_020313.1	Yang et al. (2013)
		Nautilocaris saintlaurentae	NC_021971.1	Kim et al. (2015a)
		Opaepele loihi	NC_020311.1	Yang et al. (2013)
		Rimicaris exoculata	NC_027116.1	Yu et al. (2015)
		Rimicaris kairei	NC_020310.1	Yang et al. (2013)
	Alpheidae	*Alpheus distinguendus*	NC_014883.1	Qian et al. (2011)
		Alpheus lobidens	KP276147.1	**This study**
	Atyidae	*Caridina gracilipes*	NC_024751.1	Xu et al. (2016)
		Caridina cf. *nilotica*	KU726823.1	**This study**
		Halocaridina rubra	NC_008413.1	Ivey & Santos (2007)
		Neocaridina denticulata	NC_023823.1	Yu et al. (2014)
		Paratya australiensis	NC_027603.1	Gan et al. (2015a)
	Palaemonidae	*Macrobrachium bullatum*	KM978918.1	**This study**
		Macrobrachium lanchesteri	NC_012217.1	Unpublished
		Macrobrachium nipponense	NC_015073.1	Ma et al. (2011)
		Macrobrachium rosenbergii	NC_006880.1	Miller et al. (2005)
		Palaemon carinicauda	NC_012566.1	Shen et al. (2009)
		Palaemon gravieri	NC_029240.1	Kim et al. (2015b)
		Palaemon serenus	NC_027601.1	Gan et al. (2015b)
Dendrobranchiata (outgroup)	**Sergestidae**	*Acetes chinensis*	NC_017600.1	Kim et al. (2012a)
	Penaeidae	*Farfantepenaeus californiensis*	NC_012738.1	Gutiérrez-Millán et al. (2002)
		Fenneropenaeus chinensis	NC_009679.1	Shen et al. (2007)
		Fenneropenaeus merguiensis	NC_026884.1	Zhang et al. (2016a)
		Fenneropenaeus penicillatus	NC_026885.1	Zhang et al. (2015)
		Litopenaeus vannamei	NC_009626.1	Unpublished
		Marsupenaeus japonicas	NC_007010.1	Yamauchi et al. (2004)
		Metapenaeopsis dalei	NC_029457.1	Kim et al. (2016a)
		Metapenaeus ensis	NC_026834.1	Zhang et al. (2016b)
		Parapenaeopsis hardwickii	NC_030277.1	Mao et al. (2016)
		Penaeus monodon	NC_002184.1	Wilson et al. (2000)
	Solenoceridae	*Solenocera crassicornis*	NC_030280.1	Unpublished

2 [1] *Paraglypturus tonganus* (nomen nudum)

3 [2] Mitogenome from taxonomically verified *T. australiensis* sample resubmitted as version two under same accession

4 number

List of samples for which annotations were corrected based on re-annotation with MITOS.

Accession #	Species	Genes involved†	Correction/Edits made in this study
NC_024751.1	*Caridina gracilipes*	ND2, trnS	Added ND2, removed duplicated trnS
NC_012217.1	*Macrobrachium lanchesteri*	rrnS, rrnL	Inverted coordinates for rrnS and rrnL
NC_020025.1	*Corallianassa coutierei*	trnI, trnQ	Inverted coordinates for trnI and trnQ
NC_020351.1	*Nihonotrypaea japonica*	rrnS, rrnL	Added rrnS and rrnL coordinates

2   † Genes involved in discrepancies found between NCBI's RefSeq entry and re-annotation with MITOS

Figure 1

Circular representation of three caridean and two axiidean species

These figures show composition and order of protein-coding genes (blue), ribosomal RNAs (orange), transfer RNAs (purple) and large non-coding region (grey) for the following mitogenomes: A. Alpheus lobidens, B. Caridina cf. nilotica, C. Macrobrachium bullatum, D. Callianassa ceramica, E. Trypaea australiensis.

Figure 2

Phylogenetic relationships and gene order rearrangements within Axiidea, Gebiidea and Caridea.

A. Phylogenetic tree with support values indicated at each node (top, l-r: ML and BI support for PCG (aa) + 12S + 16S dataset), bottom, l-r: ML and BI support for PCG (nt) + 12S + 16S dataset). Square brackets [ ] around a value indicate that the shown topology is in conflict with that constructed by the specific dataset. If values are absent at a node, maximum support was found for that node based on all phylogenetic inference methods and datasets. Topology shown was inferred from Bayesian analysis of PCG (aa) + 12S + 16S dataset. Codes on branches (*Gr, Pa, Ap1, Ap2, Up, Ax1, Ax2*) correspond to gene order pattern listed in B while red stars indicate mitogenomes sequenced in this study. – B. Gene order of various groups. Yellow triangles under genes indicate differences in arrangement compared to the ground pancrustacean pattern.
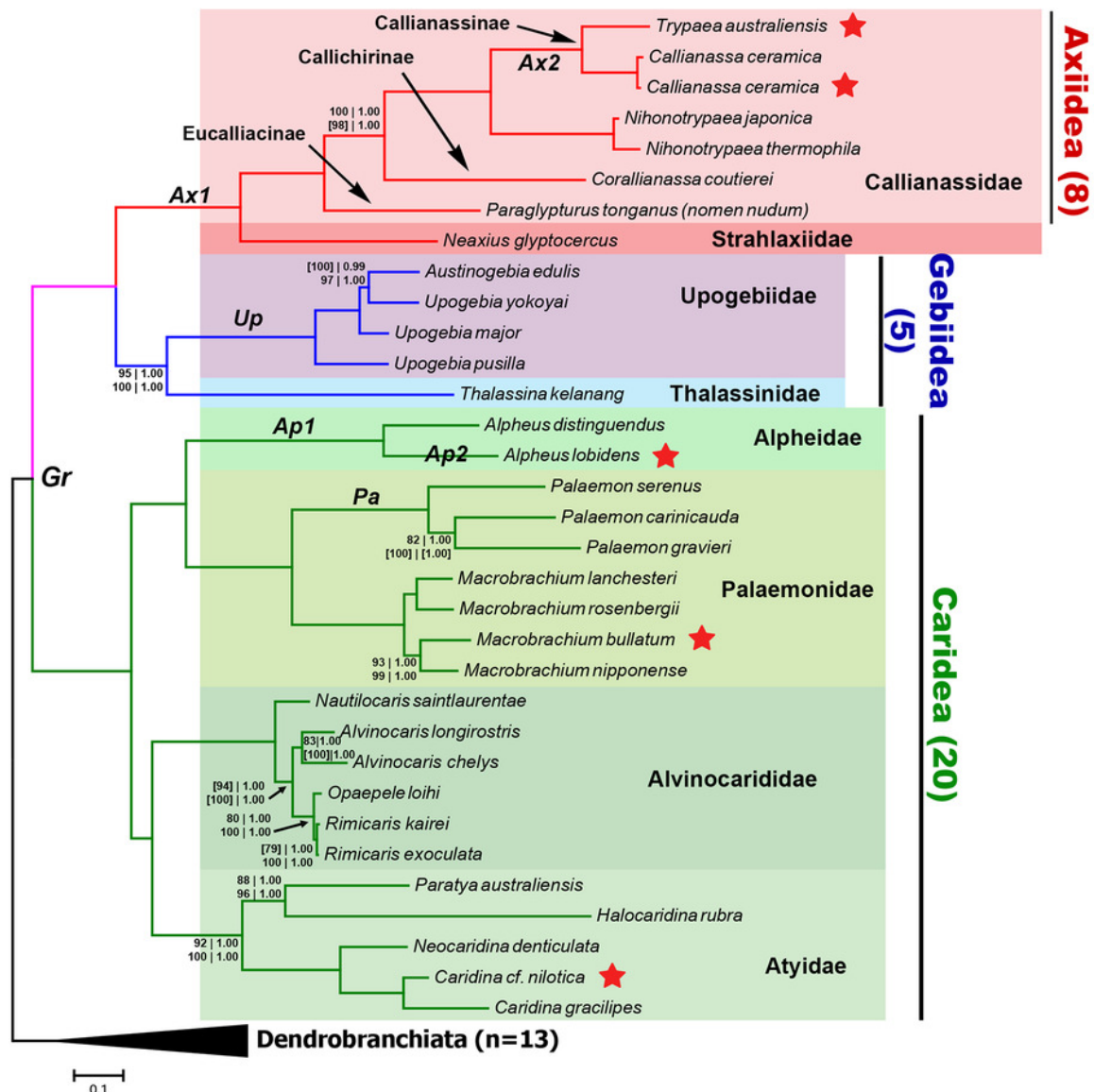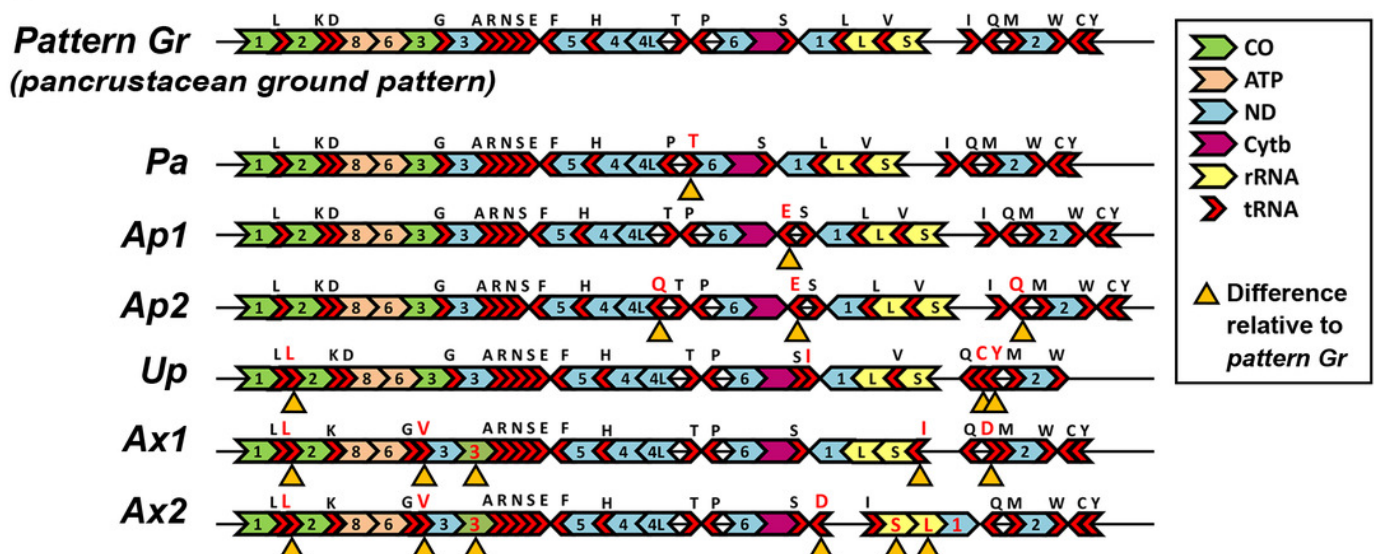
Figure 3

Relative synonymous codon usage values (RSCU) (y-axis) in protein-coding genes of mud shrimps and true shrimps

Encoded amino acid and its corresponding p-value (> or < 0.001) is shown at the top of each box plot while synonymous codons are indicated on the x-axis.

Figure 4

Patterns of variation among individuals based on RSCU values shown using multidimensional scaling (MDS) based on Euclidean dissimilarities.