Sequence comparison of the mitochondrial genomes of *Plesionika* species (Caridea: Pandalidae), Gene Rearrangement and Phylogenetic Relationships of Caridea

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Background: Despite the high number of species and ecological diversity of Caridean shrimps, there are still controversies surrounding the molecular classification of Caridea. The family Pandalidae is a diverse caridean group. However, until June 25, 2023, only nine complete mitogenomes are available in GenBank. The genus *Plesionika* from Pandalidae is considered as a polyphyletic taxon. To improve our understanding of the mitogenome evolution and phylogenetic relationships of Caridea, we present three new mitogenome sequences of genus *Plesionika* (i.e., *Plesionika ortmanni*, *Plesionika izumiae* and *Plesionika lophotes*). **Methods:** The complete mitochondrial genomes of three *Plesionika* species were sequenced using second-generation high-throughput sequencing technology. Following the assembly and annotation of the mitogenomes, structural analysis of the mitogenome was conducted, including circular maps, sequence structure characteristics, base composition, amino acid content, and frequency of synonymous codon usage. Additionally, phylogenetic analysis was performed by integrating existing mitogenome sequences of true shrimp available in GenBank. **Results:** The whole mitogenome sequences of the three *Plesionika* species consist of 37 typical genes including 13 PCGs, 22 tRNAs, 2 rRNAs and a CR, and the length of the three mitogenomes are 15,908bp (*P. ortmanni*), 16,074bp (*P. izumiae*) and 15,933bp (*P. lophotes*), respectively. We analyzed their genomic features and structural functions. Additionally, we performed selection pressure analysis on the PCGs of all Pandalidae species available in Genbank, revealing that the PCGs of Pandalidae species underwent purification selection during the process of evolution. Compared with the ancestral Caridea, the two newly sequenced *Plesionika* species (*P. izumiae* and *P. lophotes*) found the translocation of two tRNA genes, wherein *trnP* or *trnT* occurred translocation. We constructed a phylogenetic tree of Caridea using

the sequences of 13 PCGs in mitogenmoes. The results revealed that the family Pandalidae exhibited robust monophyly, while the genus *Plesionika* appeared to be a polyphyletic group. **Conclusions:** Gene rearrangements within the Pandalidae family were observed for the first time. Furthermore, a significant correlation was discovered between the phylogenetics of the Caridea clade and the arrangement of mitochondrial genes. This study provides comprehensive information on the mitogenomes of *Plesionika*, establishing a solid foundation for future research on genetic variation, systematic evolution, and breeding studies in *Plesionika*.

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Abstract

Background: Despite the high number of species and ecological diversity of Caridean shrimps, there are still controversies surrounding the molecular classification of Caridea. The family Pandalidae is a diverse caridean group. However, until June 25, 2023, only nine complete mitogenomes are available in GenBank. The genus *Plesionika* from Pandalidae is considered as a polyphyletic taxon. To improve our understanding of the mitogenome evolution and phylogenetic relationships of Caridea, we present three new mitogenome sequences of genus *Plesionika* (i.e., *Plesionika ortmanni*, *Plesionika izumiae* and *Plesionika lophotes*).

Methods: The complete mitochondrial genomes of three *Plesionika* species were sequenced using second-generation high-throughput sequencing technology. Following the assembly and

annotation of the mitogenomes, structural analysis of the mitogenome was conducted, including circular maps, sequence structure characteristics, base composition, amino acid content, and frequency of synonymous codon usage. Additionally, phylogenetic analysis was performed by integrating existing mitogenome sequences of true shrimp available in GenBank.

Results: The whole mitogenome sequences of the three *Plesionika* species consist of 37 typical genes including 13 PCGs, 22 tRNAs, 2 rRNAs and a CR, and the length of the three mitogenomes are 15,908 bp (*P. ortmanni*), 16,074 bp (*P. izumiae*) and 15,933 bp (*P. lophotes*), respectively. We analyzed their genomic features and structural functions. Additionally, we performed selection pressure analysis on the PCGs of all Pandalidae species available in Genbank, revealing that the PCGs of Pandalidae species underwent purification selection during the process of evolution. Compared with the ancestral Caridea, translocation of two tRNA genes, i.e., *trnP* or *trnT*, were found in the two newly sequenced *Plesionika* species – *P. izumiae* and *P. lophotes*. We constructed a phylogenetic tree of Caridea using the sequences of 13 PCGs in mitogenomes . The results revealed that the family Pandalidae exhibited robust monophyly, while the genus *Plesionika* appeared to be a polyphyletic group.

Conclusions: Gene rearrangements within the Pandalidae family were observed for the first time. Furthermore, a significant correlation was discovered between the phylogenetics of the Caridea clade and the arrangement of mitochondrial genes. This study provides comprehensive information on the mitogenomes of *Plesionika*, establishing a solid foundation for future research on genetic variation, systematic evolution, and breeding studies in *Plesionika*.

Subjects Aquaculture, Fisheries and Fish Science, Biodiversity, Evolutionary Studies, Genomics, Marine Biology

Keywords Pandalidae; *Plesionika*; mitochondrial genome; gene rearrangement; phylogenetic relationships

Introduction

Caridea Dana, 1852 is one of the largest infraorders within Decapoda, comprising over 3,400 species across 36 families (*De Grave and Fransen, 2011*; *Liao et al., 2017*). Caridean shrimps, with their wide distribution and varied habitats, provide an excellent model for studying the origin and adaptive evolution of aquatic organisms in different aquatic habitats (*Sun et al., 2020*). Morphological characteristics, such as pereopods and mouthparts, have traditionally been used in taxonomic studies to classify Caridean shrimps, owing to their extensive morphological variation

and diverse lifestyles, including free-living and symbiotic relationships (*Felgenhauer and Abele, 1983*; *Xu et al., 2005*). However, it remains uncertain whether this classification based on morphology reflects the phylogenetic relationships between families and superfamilies (*Ye et al., 2021*). The use of molecular sequences aids in reconstructing the phylogenetic relationships among species, effectively addressing the limitations of traditional taxonomy and resolving many controversial issues in the fields of classification and systematic evolution (*Wang et al., 2021*; *Wang et al. 2018*; *Miller et al., 2005*; *Bai et al., 2018*). Mitochondrial genomes (mitogenomes), characterized by their simple structure, rich gene content, easy extraction, maternal inheritance, high conservation , low mutation rate, and rapid gene evolution (*Boore, 1999*), have been extensively used in phylogenetic and phylogeographic analyses of animal taxa (*Gong et al., 2019*; *Elmerot et al., 2002*; *Chak et al., 2020*). Previous studies have explored the phylogenetic relationships within Caridea using molecular markers, albeit with a limited number of species. Nonetheless, debates regarding its molecular phylogeny persist. Some scholars propose that Atyidae (De Haan, 1849) represents the basal clade of Caridea (*Li et al., 2011*; *Bracken et al., 2009*), a finding not supported by other studies (*Ye et al., 2021*). Additionally, the monophyly of certain families within Caridea is contentious. Bracken et al. (2009) support the monophyly of five families: Alvinocarididae (Christoffersen, 1986), Alpheidae (Rafinesque, 1815), Crangonidae (Haworth, 1825), Pandalidae (Haworth, 1825), and Processidae (Ortmann, 1896) (*Bracken et al., 2009*). Li et al. (2011) report that the majority of Caridea, excluding Hippolytidae (Spence Bate, 1888) and Palaemonidae (Rafinesque, 1815), exhibits monophyly (*Li et al., 2011*). However, Ye et al. (2021) describe the monophyly of Hippolytidae and Palaemonidae (*Ye et al., 2021*), while Sun et al. (2020) specifically describe the monophyly of Palaemonidae (*Sun et al., 2020*). Furthermore, the phylogenetic relationship between Alvinocarididae and Atyidae has long been a topic of discussion in Caridea systematics (*Ye et al., 2021*; *Boore, 1999*; *Li et al., 2011*; *Wang et al., 2019*; *Sun et al., 2021*).

The family Pandalidae Haworth, 1825 is currently one of the largest family-level units within the infraorder Caridea, and its species possess diverse biological characteristics and lifestyles. This includes the occurrence of protandrous hermaphroditism in *Pandalus* (Leach, 1814) and *Pandalopsis* (Spence Bate, 1888) (*Liao et al., 2019*; *Butler, 1980*; *Komai, 1999*; *Bergström, 2000*), bioluminescence in *Stylopandalus* (Coutière, 1905) and *Heterocarpus* (A. Milne-Edwards, 1881) (*Herring, 1985*), and the ability to form symbiotic relationships with other

invertebrates (*Komai, 1999*; *Bruce, 1983*; *Chan, 1991*; *Crosnier, 1997*; *Horká, 2014*). Pandalidae contains 189 species across 23 genera (*De Grave, 2009*), which is widely distributed in both shallow and deep waters (*Sun et al., 2020*). Despite the ecological and economic importance of these species, the current mitogenome data available for Pandalidae are rather limited, with only 9 complete mitogenomes available in GenBank (until June 25 2023, excluding UNVERIFIED) (https://www.ncbi.nlm.nih.gov/nuccore). The genus *Plesionika* Bate, 1888, the most diverse genus in Pandalidae, comprises 93 species (*De Grave and Fransen, 2011*; *Cardoso, 2011*; *Jiang, 2018*) and is widely distributed throughout subtropical and tropical waters worldwide (*Chace and Bruce, 1985*). Until now, only two species of the genus with a complete mitogenome available in the GenBank database (i.e. *Plesionika edwardsii* (OP087601.1) and *Plesionika sindoi* (MH714453.1)). Some studies based on the partial mitochondrial sequences (*COI* and *16S rRNA*) suggest that *Plesionika* may not be monophyletic (*Silva et al., 2013*; *Chakraborty et al., 2015*; *Chakraborty et al., 2021*). The phylogenetic relationships obtained by Liao et al. (2019) (*Liao et al., 2019*), utilizing two partial fragments of mitochondrial (*12S rRNA* and *16S rRNA*) and six nuclear genes (*atpβ*, *Enolase*, *H3*, *NaK*, *PEPCK* and *GAPDH*), also indicated that *Plesionika* did not form a monophyletic group. Silva et al. (2013) (*Silva et al., 2013*) suggested that the deep-water species are paraphyletic with shallow-water species. Their study encompassed a total of seven *Plesionika* species, and phylogenetic analyses were conducted separately using the *16S rRNA* and *COI* genes. Based on the analysis results, these species were classified into two main clades. Clade I mainly consists of species distributed in shallower marine waters (< 400 meters), including *Plesionika heterocarpus*, *Plesionika scopifera*, and *Plesionika antigai*. In contrast, clade II includes species found in deeper marine waters (> 400 meters), such as *Plesionika acanthonotus*, *Plesionika narval*, *Plesionika edwardsii*, and *Plesionika martia*. The taxonomic status of various species within the genus *Plesionika* is debatable (*Crosnier, 1997*; *Komai and Chan, 2003*). The expanded availability of complete mitogenomes has the potential to aid in unraveling the phylogeny of *Plesionika*. This can be accomplished by offering multiple loci with varying rates of evolution, thus enhancing our understanding of their evolutionary relationships.

In the present study, we sequenced and analyzed three complete mitogenomes of *Plesionika* species (i.e. *Plesionika ortmanni*, *Plesionika izumiae*, and *Plesionika lophotes*). Our objectives were to (1) test the hypothesis of non-monophyly of *Plesionika* species; (2) elucidate the

taxonomic status of the Pandalidae family within Caridea; (3) investigate mitochondrial gene rearrangement patterns within Caridea; (4) examine the phylogenetic relationships within Caridean shrimps.

Materials & Methods

Sampling, identifcation and DNA extraction

Three wild species of *P. ortmanni*, *P. izumiae* and *P. lophotes*, were collected from two different areas in Zhejiang Province, China (Table 1). The specimens were morphologically identified by experts from the Marine Biology Museum of Zhejiang Ocean University, with the identification process referencing relevant literature (*Kim et al., 2012; Li, 2006*). The three *Plesionika* species share characteristics such as having no dorsal ridges or protrusions on their abdomens. Additionally, the bristles on the antennal stalk are sharp and pointed, extending to the distal edge of the first antennal segment. The differences include: in *P. ortmanni*, the sixth abdominal segment is 1.5 times its maximum height; the tail fan is 1.5 times the length of the sixth abdominal segment; there are three pairs of spines on the dorsal margin; and there are three pairs of spines on the posterior margin. The length of the antennal scale is 4.3 to 4.4 times its width. The second pair of walking legs is approximately equal in size. In *P. izumiae*, the sixth abdominal segment is 1.7 times its maximum height; the tail fan is 1.4 times the length of the sixth abdominal segment; there are three pairs of small spines on the dorsal margin; and there are three pairs of spines on the posterior margin. The length of the antennal scale is 4.2 times its width. The second pair of walking legs are unequal in size. In *P. lophotes*, the sixth abdominal segment is 1.5 times its maximum height; the tail fan is 1.6 times the length of the sixth abdominal segment; there are four pairs of small spines on the dorsal margin; and there are three pairs of spines on the posterior margin. The length of the antennal scale is approximately 3.4 times its width. The second pair of walking legs are unequal in size. Samples were preserved in absolute ethanol before DNA extraction. The total DNA was extracted using the salt-extraction procedure and stored at − 20 °C for sequencing (*Aljanabi and Martinez, 1997*).

Table 1. Sampling locations and dates for the three samples.

Mitogenomes Sequencing, Assembly, and Annotation

The complete mitogenomes of three *Plesionika* species were sequenced using next-generation sequencing (NGS) on the Illumina Hiseq X Ten platform by Origin gene Bio-pharm Technology Co. Ltd., Shanghai, China. The mitochondrial genomic DNA of the samples underwent initial quality control, where 1.0% agarose gel electrophoresis was utilized to assess the quality of the

DNA. Additionally, a nucleic acid quantifier (NanoDrop) was employed to detect the purity and concentration of the DNA. The quality-controlled mitochondrial genomic DNA of the samples was randomly fragmented into 300-500 bp segments using a Covaris M220 ultrasonic disruptor. The fragmented DNA was subsequently purified to construct sequencing libraries. The steps involved are as follows: DNA end repair, 3' adenylation, sequencing adapter ligation, and recovery of target fragments through agarose gel electrophoresis. The final sequencing libraries were generated through PCR amplification and were subsequently subjected to sequencing on the Illumina HiSeq TM platform. Trimmomatic v0.39 software was used to filter out low-quality reads, duplicate reads, sequences with a high "N" ratio, and sequencing adapter sequences (*Bolger et al., 2014*). The reads from the three species were reassembled using the NOVOPlasty assembly software (*Dierckxsens et al., 2016*). The assembled sequences were compared with other *Plesionika* species genomes in GenBank, and the *COI* and *16S rRNA* sequences were verified using NCBI BLAST (*Altschul et al., 1997*). Aberrant start and stop codons were identified by comparing with similar codons in other invertebrate species. The online software MITOS (*Bernt et al., 2013*) was utilized for structural and functional annotation, with manual corrections performed to obtain the final complete mitogenome. The sequenced mitogenomes were uploaded to the GenBank database (Table 1).

Sequence Analysis

The circular visualization of the mitogenomes of the three *Plesionika* species was completed using the CGView server (*Grant and Stothard, 2008*). The nucleotide composition of the whole mitogenome, protein-coding genes (PCGs), rRNA, tRNA genes, and AT content were analyzed using MEGA-X (*Kumar, 2018*). The base skew values were calculated using the formulas AT-skew = (A − T)/(A + T) and GC-skew = (G − C)/(G + C) (*Alexandre et al., 2005*). To confirm the accuracy of transfer RNA genes and their secondary structures using the MITOS. (*Bernt et al., 2013*). Base composition, nucleotide composition and relative synonymous codon usage (RSCU) of each protein-coding gene were calculated using MEGA-X (*Kumar, 2018*). The Ks/Ks ratios for the three mitogenomes were estimated using DnaSP 6.0 (*Rozas et al., 2018*).

Gene Order Analysis

In addition to the three mitogenomes sequenced in this study, we obtained an additional 79 complete mitogenomes of Caridea from GenBank (Table S1) for comparative analyses. The gene arrangements of all 82 mitogenomes were compared with the ancestral Decapoda, with the aim

of identifying potential novel gene orders that have not been reported in previous studies. To ensure that observed gene order differences were not caused by mis-annotations, any mitogenomes in Caridea that deviated from the ancestral pattern underwent re-annotation using MITOS (*Bernt et al., 2013*).

Phylogenetic Analysis

To explore the phylogenetic relationships of Pandalidae, sequences of 79 species from 11 families within Caridea were downloaded from GenBank (Table S1). The mitogenomes of *Hemigrapsus sinensis* (NC_065995) and *Helicana japonica* (NC_065158) from Brachyura served as outgroup, and phylogenetic analyses were performed based on the 13 PCGs of these 84 species. The sequence of 13 PCGs from each sample were identified using DAMBE 7 software (*Xia, 2018*). The PCG sequences of these 81 species were aligned with MEGA-X's ClustalW (*Kumar, 2018*). Subsequently, Gblocks v.0.91b REF was applied to determine and select conservative regions, removing divergent and ambiguously aligned blocks (*Castresana, 2000*). DAMBE 7 was used to assess the suitability of these sequences for phylogenetic tree construction (*Xia, 2018*).

We employed the maximum likelihood (ML) method using the program IQ-tree 2.1.3 (*Minh et al., 2020*) and the Bayesian inference (BI) method using the program MrBayes 3.2.7a (*Ronquist et al., 2012*) to analyze the phylogenetic relationships. ML tree building using the program IQ-TREE (*Minh et al., 2020*), filtering the best substitution model (TIM2+F+R7) based on the Bayesian information criterion (BIC) using ModelFinder (*Kalyaanamoorthy et al., 2017*) calculations, and setting the number of boot copies 1000 ultra-fast bootstraps to rebuild the consensus tree. Using the program MrBayes v3.2 for BI construction, the BI tree model measurement firstly model testing first used PAUP 4 (*Swofford et al., 1993*) for format conversion, and then combines PAUP 4, ModelTest 3.7 (*Darriba et al., 2020*) and MRModelTest 2.3 (*Nylander, 2004*) software in MrMTgui to determine the best alternative model (GTR + I + G) according to the Akaike information criterion (AIC). Four Markov Chain Monte Carlo (MCMC) chains were simultaneously run for 2 million generations, with a sampling frequency of every 1000 generations. During the initial burn-in phase, 25% of trees were discarded, and convergence of independent runs was evaluated by the mean standard deviation of the splitting frequency (< 0.01). Finally, the phylogenetic tree was edited using the software FigTree v1.4.3 (FigTree, Version 1.4.3, 2016).

Both ML and BI were used to construct phylogenetic trees. The non-parametric bootstrap support values and Bayesian posterior probabilities generated by the two methods represent the support rates of the nodes, respectively. The non-parametric bootstrap method tends to underestimate the support rates of nodes, whereas the Bayesian method tends to overestimate them (*Suzuki et al., 2002*). A maximum likelihood value greater than 70% indicates that the clade relationship is well resolved. Conversely, a value between 50% and 70% is considered weak support, and anything below is regarded as unresolved (*Huelsenbeck et al., 1993*). Similarly, a Bayesian posterior probability of 95% or higher indicates that the clade support rate is well established (*Leaché et al., 2002*).

Results

Genome structure, composition, and skewness

The mitogenomes of the three *Plesionika* species consist of 15,908 bp (*P. ortmanni*), 16,074 bp (*P. izumiae*) and 15,933 bp (*P. lophotes*). The GenBank accession numbers are OP650932, OP650933 and OP650934, respectively (Fig. 1). These mitogenomes exhibit a closed, circular, double-stranded DNA structure and encompass a total of 37 genes, including 13 PCGs, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and a control region (CR). Notably, 14 genes were localized on the light chain, comprising 4 PCGs (*ND5*, *ND4*, *ND4L*, and *ND1*), 8 tRNA genes (*trnF*, *trnH*, *trnP*, *trnL1*, *trnV*, *trnQ*, *trnC* and *trnY*), and 2 rRNA genes (*16S rRNA* and *12S rRNA*). Conversely, the remaining 23 genes were situated on the heavy chain (Fig. 1, Table S2). The CR was located between *12S rRNA* and *trnI* in all three species, with *P. ortmanni* being the longest (472 bp) and *P. izumiae* being the shortest (68 bp) (Table S2).

The nucleotide compositions of the three newly sequenced mitogenomes were within the following ranges: A: 32.70% to 35.91%, T: 31.46% to 31.94%, G: 11.60% to 13.85%, C: 20.55% to 21.98% (Fig. 2A). The contents of A and T exhibited high values, indicating that codon usage was biased towards A and T, which is consistent with the reported complete Pandalidae mitogenomes (*Sun et al., 2020*). The three species had low G and C contents, indicating obvious bias against G and C. Among them, the AT contents ranged from 64.16% to 67.85%, while the AT-skew were positive in the range of 0.019 ~ 0.058 and GC-skew were negative in the range of − 0.227 ~ − 0.285 (Fig. 2B).

Figure 1. Complete mitogenome map of three *Plesionika* species.

Figure 2. Nucleotide composition (A) and nucleotide skews (B) of the three newly sequenced *Plesionika* species mitogenomes.

Protein-coding genes and codon usage

The PCGs in the three *Plesionika* species mitogenomes had total length of 11,192 bp (*P. ortmanni*), 11,134 bp (*P. izumiae*) and 11,041 bp (*P. lophotes*), respectively, including 7 NADH dehydrogenases (*ND1-6* and *ND4L*), 3 cytochrome oxidases (*COI-III*), 2 ATPases (*ATP6* and *ATP8*) and 1 cytochrome b (*Cytb*) (Fig. 1, Table S2). The longest PCG of these species was the *ND5* at 1644 to 1719 bp and the shortest was the *ATP8* gene at the same length of only 159 bp. The high AT contents were also observed in the base composition of these species, with the highest AT content founded in *P. lophotes* at 66.05 %, while the AT-skew were negative in the range of − 0.170 ~ − 0.178 (Fig. 2). Upon comparison of initiation and termination codons of all PCGs of the three *Plesionika* species, we found five initiation codons and two termination codons. The PGCs of the three mitogenomes were mostly initiated with ATG, ATT and ATA, only the *ATP8* of *P. ortmanni* and *ND3* of *P. lophotes* start with ATC, and the *COI* of *P. ortmanni* start with ACG (Table S3). The majority of the PGCs of the three mitogenomes were terminated with TAA and TAG, while the *ND4* of three mitogenomes, the *COI* of *P. ortmanni* and the *ND5* of *P. lophotes* stop with single T. Incomplete termination codons are a remarkably common phenomenon in mitochondrial genes of vertebrates and invertebrates (*Hamasaki et al., 2017*).

The analysis of the three species showed that the amino acid compositions in PCGs were relatively similar (Fig. 3, Table S4). The most frequently used amino acids are Asn, Leu1, Lys, Phe, Pro and Thr, while Arg and Cys are the least common amino acids. Comparing the relative synonymous codon usage (RSCU) of 13 PCGs in the three species, the result showed that the usage frequency of UUA (Leu), UCU (Ser) and AUA (Met) codons in their sequenced mitogenomes was higher. In *P. ortmanni*, the highest RSCU was found for CGA (Arg), followed by UUA (Leu), ACU (Thr), UCU (Ser) and AUA (Met). In *P. izumiae*, the highest RSCU was found for UUA (Leu), followed by AUA (Met), CAA (Gln), UCU (Ser) and CCU (Pro). And in *P. lophotes*, the highest RSCU was found for CGA (Arg), followed by UUA (Leu), UCU (Ser), CCU (Pro) and AUA (Met). The lowest RSCU in all three species was observed for GAG (Glu).

Figure 3. The frequency of mitochondrial PCG amino acids (A) and relative synonymous codon usage (RSCU) (B) of three newly sequenced *Plesionika* mitogenomes.

Transfer and Ribosomal RNAs

In common with other Caridea mitogenomes, the mitogenome of the three *Plesionika* species contains 22 tRNA genes (Fig. 1, Table S2). The total length of the tRNAs in the three *Plesionika*

species mitogenomes were 1471 bp (*P. ortmanni*), 1465 bp (*P. izumiae*) and 1476 bp (*P. lophotes*), and the length of the tRNAs in these species ranging from 59 to 72 bp (Table S2). All of the tRNAs showed high AT contents, with an AT content of the three species were 66.96% (*P. ortmanni*), 66.21% (*P. izumiae*), 67.48% (*P. lophotes*) (Fig. 2A). The tRNA genes of *P. ortmanni* had a weakly negative AT skew (− 0.005) and positive GC skew (0.115), while the tRNA genes of *P. izumiae* and *P. lophotes* had positive AT skew (0.021 and 0.034, respectively) and GC skew (0.083 and 0.079, respectively) (Fig. 2B). The secondary cloverleaf structure of the 22 tRNAs from these species were examined. In *P. ortmanni*, *trnS1* cannot form a secondary structure due to the lack of dihydrouracil (DHU) arms, and this phenomenon is common in metazoans (*Yamauchi et al., 2003*) (Fig. 4). Additionally, the *trnP* gene in *P. ortmanni*, the *trnA* gene in *P. izumiae*, and the *trnD*, *trnF*, and *trnH* genes in *P. lophotes* lacked the TΨC loop, while the remaining genes exhibited a typical cloverleaf structure (*Rich and Rajbhandary, 1976*). Comparing the tRNA genes of the three species, it was found that each corresponding amino acid was encoded by the same anticodon.

The total lengths of the *16S rRNA* and *12S rRNA* genes were similar in the three species, with *P. ortmanni*, *P. izumiae*, and *P. lophotes* having total lengths of 1361 bp, 1328 bp, and 1322 bp for *16S rRNA*, and 803 bp, 811 bp, and 812 bp for *12S rRNA*, respectively (Table S2). The *16S rRNA* and *12S rRNA* genes of three species were located between *trnL1* and *trnI*, and were separated by *trnV*. These also showed high AT contents, with an AT content of the three species were 67.83% (*P. ortmanni*), 69.98% (*P. izumiae*), 70.76% (*P. lophotes*) (Fig. 2A). The rRNA genes of *P. ortmanni* had a weakly positive AT skew (0.012) and positive GC skew (0.253), while both the rRNA genes of *P. izumiae* and *P. lophotes* had negative AT skew (− 0.059 and − 0.056, respectively) and positive GC skew (0.349 and 0.339, respectively) (Fig. 2B).

Figure 4. The predicted secondary structure of tRNA genes, from *trnA* to *trnW*. The nucleotide substitution pattern of tRNA genes in three newly sequenced *Plesionika* mitogenomes has been exhibited with the reference species *P. ortmanni*.

Selective Pressure Analysis

In genetics, the Ka/Ks ratio, which represents the ratio between the nonsynonymous substitution sites (Ka) and the synonymous substitution sites (Ks), is commonly used to understand the dynamic evolution of PCGs. In this study, the Ka/Ks ratios of the 13 PCGs were calculated using the 9 sequenced Pandalidae species (Table S1) to investigate the relationship between evolution and selection pressure (Fig. 5). The results showed that the Ka/Ks ratios of the PCGs range from

0.088 for *COI* to 0.341 for *ATP8*. The Ka/Ks ratio of the *COI* gene was the lowest, indicating that the *COI* gene was under the greatest selection pressure and the gene sequence was relatively conservative. As a result, it is widely used as a potential molecular marker in species identification and phylogenetic studies (*Astrin et al., 2016*).

In general, a gene is considered to be positively selected when the Ka/Ks is greater than 1, neutral evolutionary when the Ka/Ks is equal to 1, and purified selected when the Ka/Ks is less than 1 (*Nei and Kumar, 2000*; *Yang, 2006*). In this study, the Ka/Ks ratios of the 13 PCGs genes were less than 1, indicating that the genes of the Pandalidae species were subjected to purification selection during evolution.

Figure 5. Selective pressure analysis for 13 PCGs among 12 Pandalidae mitochondrial genomes. Species of Pandalidae are shown in Table S1.

Gene Rearrangement

Mitochondrial gene arrangement is an important tool for the study of systematic geography and phylogeny, which provides informative insights into the evolution among metazoans (*Beagley et al., 1998*; *Searle, 2000*). In general, mitochondrial gene arrangement is relatively stable in vertebrates, such as fish, amphibians, and most mammals (*Fu et al., 2009*). However, in invertebrates, varying degrees of gene rearrangement are commonly observed in mitogenomes (*Ye et al., 2021*; *Boore, 1999*). In this study, we compared the gene orders of the infraorder Caridea mitogenomes with ancestral Decapoda, the mitochondrial gene orders (MGOs) of the family Atyidae, Alvinocarididae, Acanthephyridae (Spence Bate, 1888), Oplophoridae (Dana, 1852) and Nematocarcinidae (Smith, 1884) were identical to those of the ancestral Decapoda, and the gene rearrangement was found in 24 species in 6 family of Caridea (Fig. 6). This contradicts the previous view that the gene order in the Caridea is conservative (*Wang et al. 2018*; *Miller et al., 2005*; *Ivey and Santos, 2007*; *Lü et al., 2019*).

Compared with the ancestral Caridea, the MGOs of the newly sequenced *P. ortmanni* and the other 9 Pandalidae species from GenBank (i.e. *Bitias brevis*, *Chlorotocus crassicornis*, *Heterocarpus ensifer*, *Heterocarpus sibogae*, *Pandalus borealis*, *Pandalus prensor*, *Parapandalus sp.*, *P. edwardsii*, *P. sindoi*) remained consistent with the ancestral gene order (*Sun et al., 2020*). Conversely, the two newly sequenced *Plesionika* species (*P. izumiae* and *P. lophotes*) have a translocation, for which the gene order is *trnK* - *trnD* instead of *trnD* - *trnK*. In Alpheidae, gene rearrangement was observed in *Leptalpheus forceps* and 7 *Alpheus* (Fabricius, 1798) species, in which *trnE* translocated and inverted with *trnP* (*Ye et al., 2021*; *Shen et al.,*

2012). In addition, *Alpheus lobidens* has an additional *trnQ* repeat downstream of *ND4L* (*Wang et al., 2019*). In Palaemonidae, 9 *Palaemon* (Weber, 1795) species were found the translocation of two tRNA genes, wherein *trnP* or *trnT* occurred translocation (*Shen et al., 2009*). While the MGOs of *Hymenocera picta* occurred a novel order, the gene fragment (*ND1 - trnL1 - 16S rRNA - trnV - 12S rRNA - trnI - trnQ*) was moved from the downstream of *trnS2* to the position downstream of *ND4L* (*Ye et al., 2021*). In Lysmatidae (Dana, 1852), both *Exhippolysmata ensirostris* and *Lysmata vittata* experienced gene translocation. Specifically, the *trnL2* of *E. ensirostris* translocates and inverses with *COII* (*Ye et al., 2021*), and the *trnA* of *L. vittata* translocates and inverses with *trnR*. The *trnC* of *Saron marmoratus* in Hippolytidae was rearranged from the downstream of *trnW* to the position downstream of *trnQ*. Moreover, the MGOs of *Thor amboinensis* in Thoridae (Kingsley, 1878) have undergone significant changes. Specifically, the *trnQ*, *trnT,* and a gene fragment comprising (*ND6 - Cytb - trnS2*) were rearranged from the downstream of *trnI*, *ND4* and *trnP*, respectively, to form a new gene fragment (*trnQ - trnT - ND6 - Cytb - trnS2*) downstream of *trnS1*. Additionally, the *trnP* was moved from downstream of *trnT* to the position downstream of *12S rRNA*, the *trnC* was relocated from the downstream of *trnW* to the position downstream of *trnI*, the *trnM* translocated and inverted with *ND2*, and the *trnY* was relocated from the downstream of *trnC* to the position downstream of *16S rRNA*.

In this study, we report the first instances of gene rearrangement in Pandalidae, as observed in the mitogenomes of two newly sequenced *Plesionika* species (*P. izumiae* and *P. lophotes*). This discovery revealed two distinct gene arrangement patterns in the genus *Plesionika*, thereby highlighting the non-conservative nature of gene arrangement in the Pandalidae family. In the future, with the continuous increase in mitochondrial genomic data of the Pandalidae, gene arrangement may provide important evolutionary information for inferring the phylogenetic relationships within the Pandalidae and between the Pandalidae and other groups of Caridea.

Figure 6. Linear representation of the mitochondrial gene arrangement of the ancestral mitogenome of pancrustaceans and Caridea species. In this study, the three newly sequenced species are marked with blue box.

Phylogenetic Relationships

In this study, we constructed a phylogenetic tree of Caridea using the sequences of 13 PCGs in mitogenomes. Our analysis included 82 caridean species, with *P. ortmanni*, *P. izumiae*, and *P. lophotes* as focal species, using *H. sinensi* and *H. japonica* as outgroups. The topological

structures of the phylogenetic tree reconstructed using two methods are identical (Fig. 7), but there were slight differences in the support values of some of the clade branches. The support values of BI were generally higher than ML, with the majority of the nodes having a support value of 1. On the other hand, the support values of ML, except for one node with a support value of 53 (the node between *Alpheus japonicus* and *Alpheus randalli*), were between 78 to 100 for all other nodes. Phylogenetic tree analysis showed that all families within Caridea are monophyletic. The families of Pandalidae, Thoridae, Lysmatidae, and Hippolytidae exhibited strong monophyly and clustered together as a single clade. Acanthephyridae and Oplophoridae were closely related in phylogenetic relationship, forming a sister group, and subsequently clustered with Alvinocarididae. These three families then formed a large clade with Nematocarcinidae. Additionally, the families of Alpheidae and Palaemonidae were also found to be closely related, forming a sister group relationship.

The phylogenetic tree revealed that the three newly sequenced species within the family Pandalidae did not cluster together. Phylogenetic analysis showed that *P. izumiae* and *P. lophotes* were closely related and formed a distinct branch, while *P. ortmanni*, *P. sindoi* and *P. edwardsii* clustered together on another separate branch. At the genus level, we observed that the genera *Plesionika* and *Heterocarpus* within the family Pandalidae were not monophyletic. The five species of the genus *Plesionika* formed two distinct clades, while *Parapandalus sp*. was grouped with the genus *Heterocarpus*.

Figure 7. The phylogenetic tree based on 13 PCGs was inferred using Bayesian inference (BI) and maximum likelihood (ML) methods. The number at each clade is the bootstrap probability and the three newly sequenced species are marked with red dots.

Discussion

A comparison of the base content of the mitochondrial genomes of three *Plesionika* species revealed that all three genomes showed a higher AT base content than CG, a phenomenon commonly observed in the mitochondrial genome sequences of decapods (*Sun et al., 2020; Zhu et al., 2021; Wang et al., 2019*). The *trnS1* gene of the *P. ortmanni* lacked the DHU arm, preventing it from forming a typical cloverleaf structure. The loss of the DHU arm in the *trnS* gene has been reported in most mitochondrial genome studies of Caridean shrimp (*Ye et al., 2021; Shen et al., 2012*). Selective pressure analysis was conducted separately on the PCGs of nine species in the Pandalidae family, and the results showed that the Ka/Ks values of all 13 PCGs were less than 1. Zhu et al. (2021) previously performed a selective pressure analysis on

415 species from the Palaemonidae family within the Caridea and also concluded that the Ka/Ks
416 values of all 13 PCGs were less than 1 (*Zhu et al., 2021*).
417 The phylogenetic tree results indicated that the genera *Plesionika* and *Heterocarpus* within
418 the family Pandalidae were not monophyletic. This finding is consistent with a previous study by
419 Wang et al. (2021) (*Wang et al., 2021*) and Liao et al. (2019) (*Liao et al., 2019*), who used
420 mitogenome genes or nuclear genes to construct phylogenetic trees and examine the
421 phylogenetic relationships within the family Pandalidae. Their results supported that the genera
422 *Plesionika* and *Heterocarpus* are polyphyletic The five *Plesionika* species included in our study
423 were also assigned to two different clades in their results. Additionally, several studies based on
424 partial mitochondrial sequences (*COI* and *16S rRNA*) have supported the non-monophyly of
425 *Plesionika* (*Silva et al., 2013*; *Chakraborty et al., 2015*; *Chakraborty et al., 2021*). This result
426 may be attributed to morphological differences among species within the genus *Plesionika*,
427 especially the distinct asymmetry of the second pereiopod in *P. izumiae* and *P. lophotes*
428 compared to other *Plesionika* species. At the family level, Pandalidae exhibits strong
429 monophyly, which is consistent with previous research findings (*Sun et al., 2020*; *Ye et al., 2021*;
430 *Wang et al., 2021*; *Chak et al., 2020*; *Sun et al., 2021*; *Cronin et al., 2022*). Regarding
431 phylogenetic relationships among families, the families Acanthephyridae, Oplophoridae, and
432 Alvinocarididae are closely related and form sister groups; similarly, the families Alpheidae and
433 Palaemonidae are also closely related and form sister groups. These results are consistent with
434 previous phylogenetic studies (*Chak et al., 2020*; *Sun et al., 2021*; *Cronin et al., 2022*). While
435 our phylogenetic tree topology is consistent with previous research, there are some differences.
436 Specifically, our results conflict with those of Li et al. (2011), who suggested that Atyidae
437 represent basal lineages within the Caridea based on five nuclear genes (*Li et al., 2011*).
438 Similarly, Bracken et al. (2009) (*Bracken et al., 2009*) inferred that Atyidae represent basal
439 lineages within Caridea based on both mitochondrial and nuclear genes. Moreover, Li et al.
440 (2011) (*Li et al., 2011*) found that members of the families Palaemonidae and Hippolytidae did
441 not form monophyletic groups. Their study suggested that members of Hymenoceridae and
442 Gnathophyllidae clustered within the Palaemonidae clade, and *Lysmata amboinensis* of
443 Hippolytidae showed a close relationship with *Janicea antiguensis* of Barbouriidae. However,
444 according to the latest records from WoRMS, both Hymenoceridae and Gnathophyllidae have
445 been updated to Palaemonidae (*De Grave et al.,2015*), and *Lysmata amboinensis* has also been

corrected from Hippolytidae to Lysmatidae (*De Grave et al., 2011*). Therefore, our study supports that Palaemonidae and Hippolytidae are monophyletic groups. This highlights the importance of incorporating molecular techniques into species identification and classification, as they demonstrate the limitations of past morphology-based species taxonomy.

In previous studies, some scholars have suggested that mitochondrial gene arrangement could be used as a new molecular marker to assist phylogenetic analysis (*Zhang et al., 2019*; *Tan et al., 2018*; *Wang et al., 2019*). Some scholars have also recognized the potential of mitochondrial rearrangement as a "super" feature for estimating arthropod phylogenetic (*Boore et al., 1998*; *Dowton and Austin, 1999*; *Dowton et al., 2002*; *Tan et al., 2017*). Our study further analyzed the relationship between mitochondrial gene arrangement and phylogenetics in Caridea. The phylogenetic tree showed a very clear correlation between both gene arrangement and phylogenetic, and the species having gene rearrangement within each family were clustered together. In the Pandalidae family, the newly sequenced species *P. izumiae* and *P. lophotes* exhibited the same gene rearrangement and were closely related in the tree, while three *Plesionika* species (*P. sindoi*, *P. edwardsii*, and *P. ortmanni*) with the ancestral gene order formed a separate branch. This suggests that the polyphyly phenomenon of *Plesionika* may be associated with differences in gene order. In Alpheidae, both the *Alpheus* and *Leptalpheus* (Williams, 1965) genus underwent gene rearrangement and were clustered together with high support. In Palaemonidae, the species of the *Palaemon* genus were clustered together, and except for *Palaemon modestus*, all other species under *Palaemon* underwent the same gene rearrangement, while *H. picta*, which experienced different gene rearrangement, was alone in another cluster. In Lysmatidae, the species with gene rearrangement, *L. vittata* and *E. ensirostris*, were also clustered together. Overall, the six families (Pandalidae, Palaemonidae, Alpheidae, Thoridae, Lysmatidae, Hippolytidae) with gene rearrangements were clustered at the base of the Caridea phylogenetic tree, while the five families sharing the same gene order pattern formed a terminal clade. The results of this study show that there is a certain correlation between the phylogenetics of Caridea and the sorting of mitochondrial genes, but additional mitogenomes data are needed to support this result and further investigate their relationship.

Conclusions

We sequenced the complete mitogenomes of three *Plesionika* species and analyzed the basic characteristics of these mitogenomes. It was found that these genomes were relatively similar in

terms of size, nucleotide composition, and codon usage preference, but exhibited slight structural differences. Additionally, all 13 PCGs in the 12 species of the Pandalidae family underwent purifying selection, with the *COI* gene experiencing the highest selection pressure, indicating its suitability as an optimal molecular marker for species identification and phylogenetic studies within the Pandalidae. Furthermore, gene rearrangements in the Pandalidae were observed for the first time, translocation of two tRNA genes, i.e., *trnP* or *trnT*, were found in the two newly sequenced *Plesionika* species – *P. izumiae* and *P. lophotes*. Phylogenetic analysis revealed a high level of monophyly within the Pandalidae family, but the *Plesionika* genus appeared to be polyphyletic. By combining the results of gene rearrangements and phylogenetic analysis, a correlation was discovered between the phylogenetics of Caridea and the arrangement of mitochondrial genes. Families that underwent gene rearrangements were located at the base of the Caridea phylogenetic tree, while families without gene rearrangements clustered together at the terminal branch of the phylogenetic tree. This study provides extensive information regarding the mitogenomes of *Plesionika*, laying a solid foundation for future research on genetic variation, systematic evolution, and breeding of *Plesionika* using mitogenomes.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Yuman Sun: Data curation (equal); Writing – original draft (equal). Jian Chen: Data curation (equal); Writing – original draft (equal).
- Xinjie Liang: Methodology (equal); Resources (equal).
- Jiji Li: Methodology (equal); Resources (equal).
- Yingying Ye: Data curation (supporting); Funding acquisition (lead); Supervision (lead); Writing – review & editing (lead).
- Kaida Xu: Data curation (supporting); Funding acquisition (lead); Supervision (lead); Writing – review & editing (lead).

Data Deposition

All mitogenome sequences data were deposited in Genbank with accession number OP650932 (*Plesionika ortmanni*) (https://www.ncbi.nlm.nih.gov/nuccore/OP650932), OP650933 (*Plesionika izumiae*) (https://www.ncbi.nlm.nih.gov/nuccore/OP650933) and OP650934 (*Plesionika lophotes*) (https://www.ncbi.nlm.nih.gov/nuccore/OP650934).

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Figure 1

Complete mitogenome map of three *Plesionika* species.

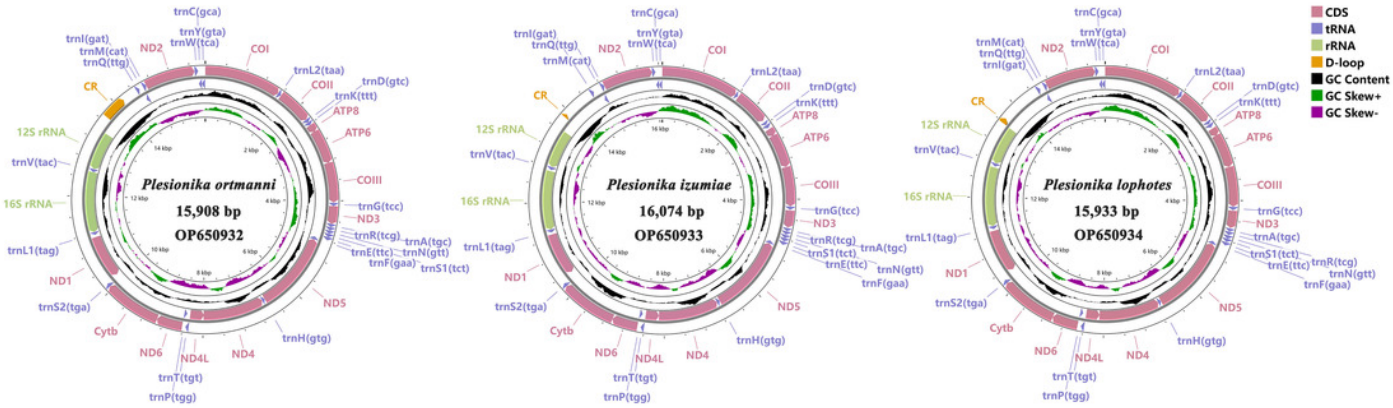


Figure 2

Nucleotide composition (A) and nucleotide skews (B) of the three newly sequenced *Plesionika* species mitogenomes

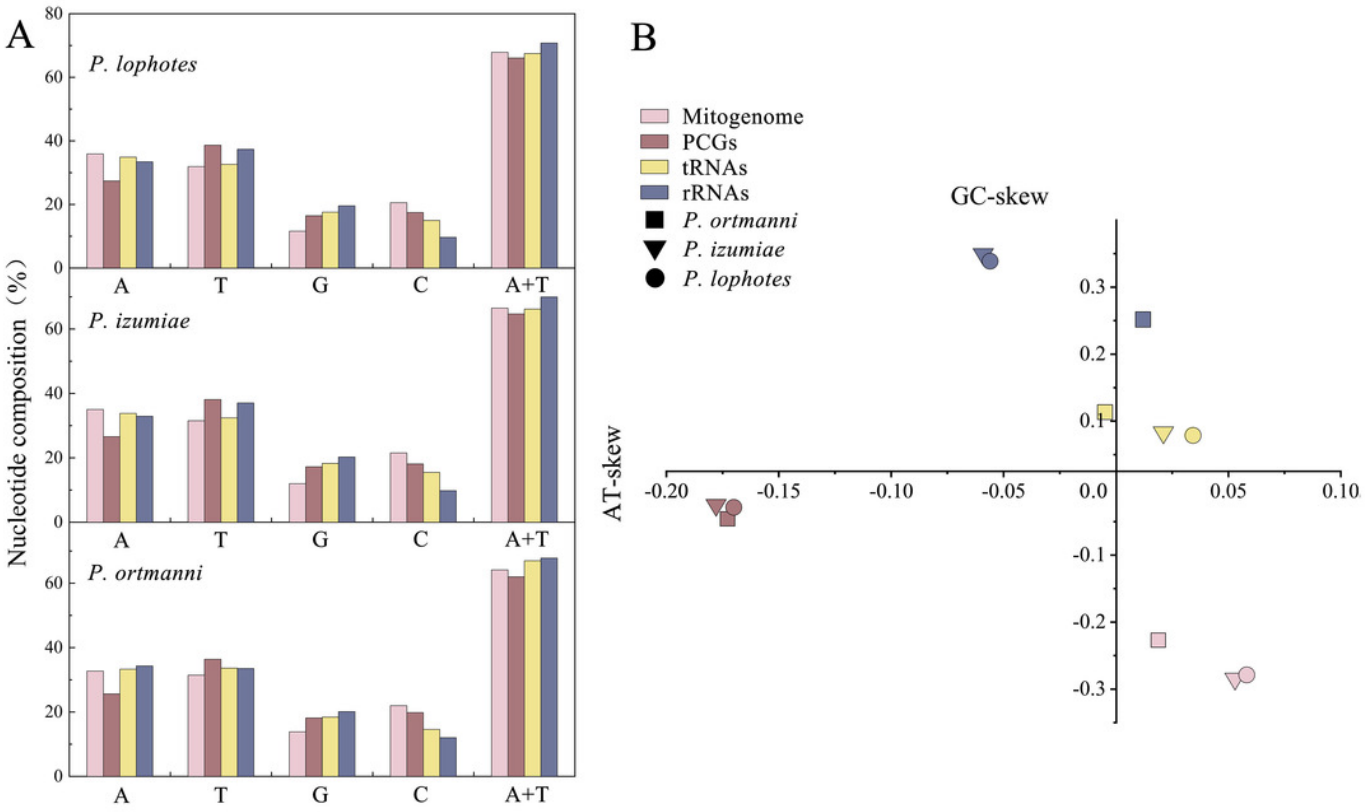


Figure 3

The predicted secondary structure of tRNA genes, from *trnA* to *trnW*. The nucleotide substitution pattern of tRNA genes in three newly sequenced *Plesionika* mitogenomes has been exhibited with the reference species *P. ortmanni*.

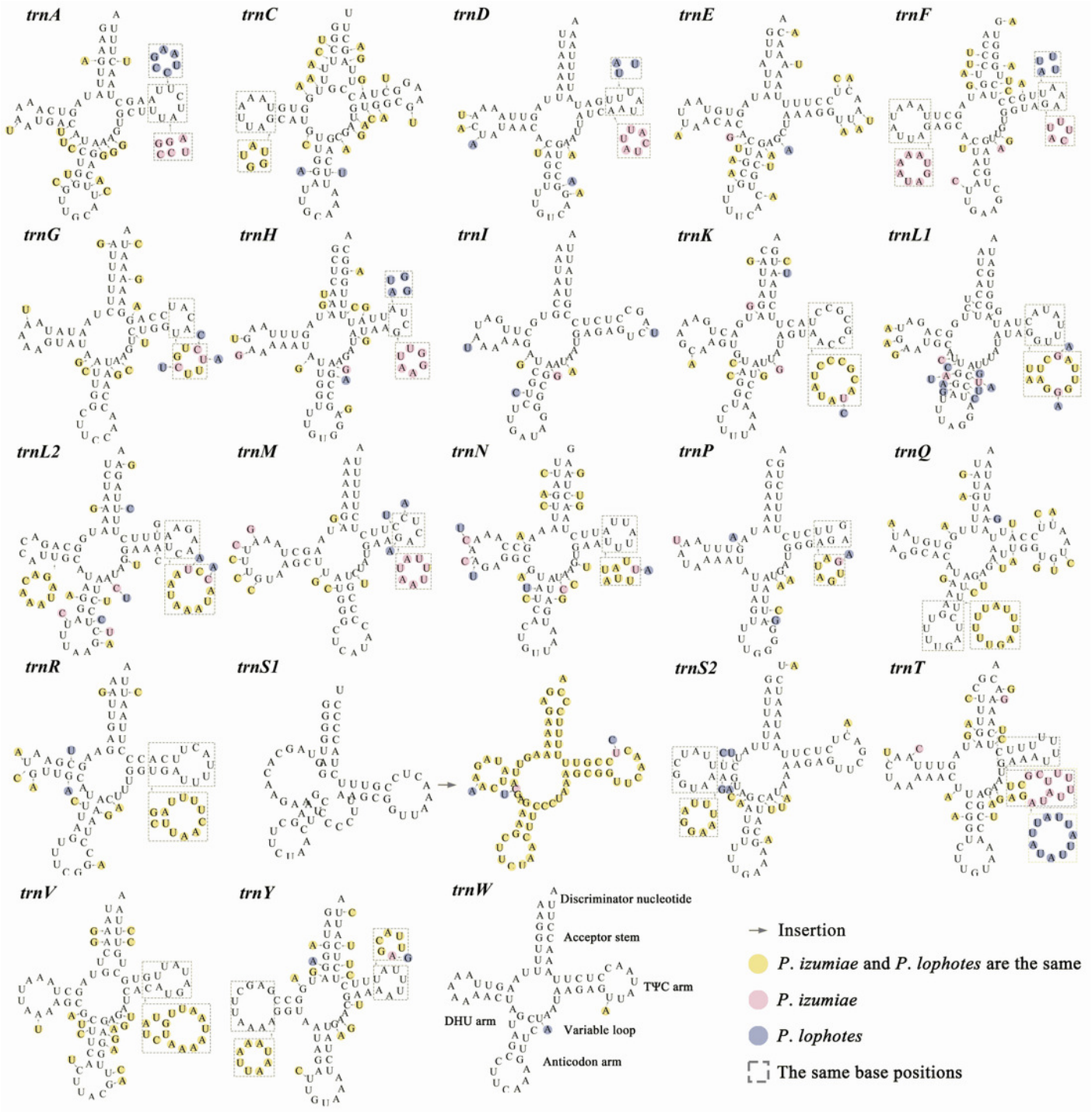

trnA
trnC
trnD
trnE
trnF
trnG
trnH
trnI
trnK
trnL1
trnL2
trnM
trnN
trnP
trnQ
trnR
trnS1
trnS2
trnT
trnV
trnY
trnW
Discriminator nucleotide
Acceptor stem
TΨC arm
DHU arm
Variable loop
Anticodon arm
Insertion
P. izumiae and P. lophotes are the same
P. izumiae
P. lophotes
The same base positions

Figure 4

Selective pressure analysis for 13 PCGs among 12 Pandalidae mitochondrial genomes. Species of Pandalidae are shown in Table S1.

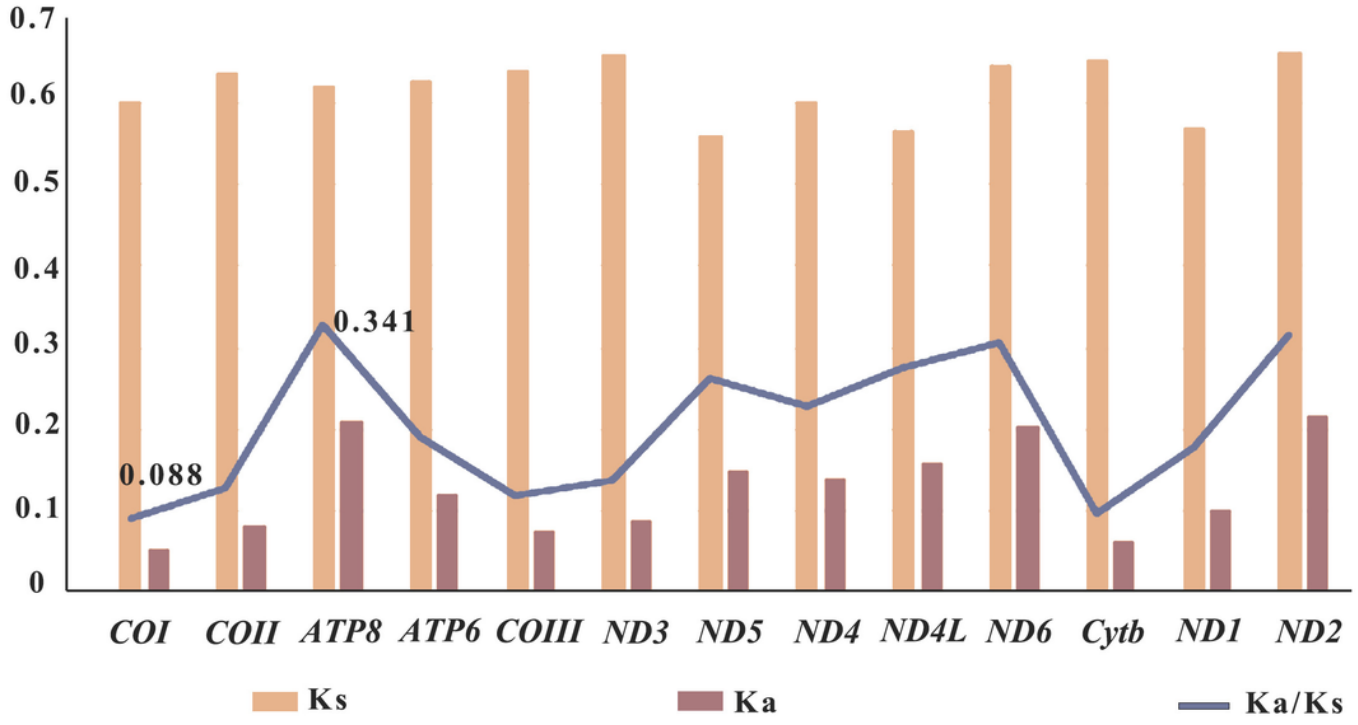


Figure 5

The frequency of mitochondrial PCG amino acids (A) and relative synonymous codon usage (RSCU) (B) of three newly sequenced *Plesionika* mitogenomes.

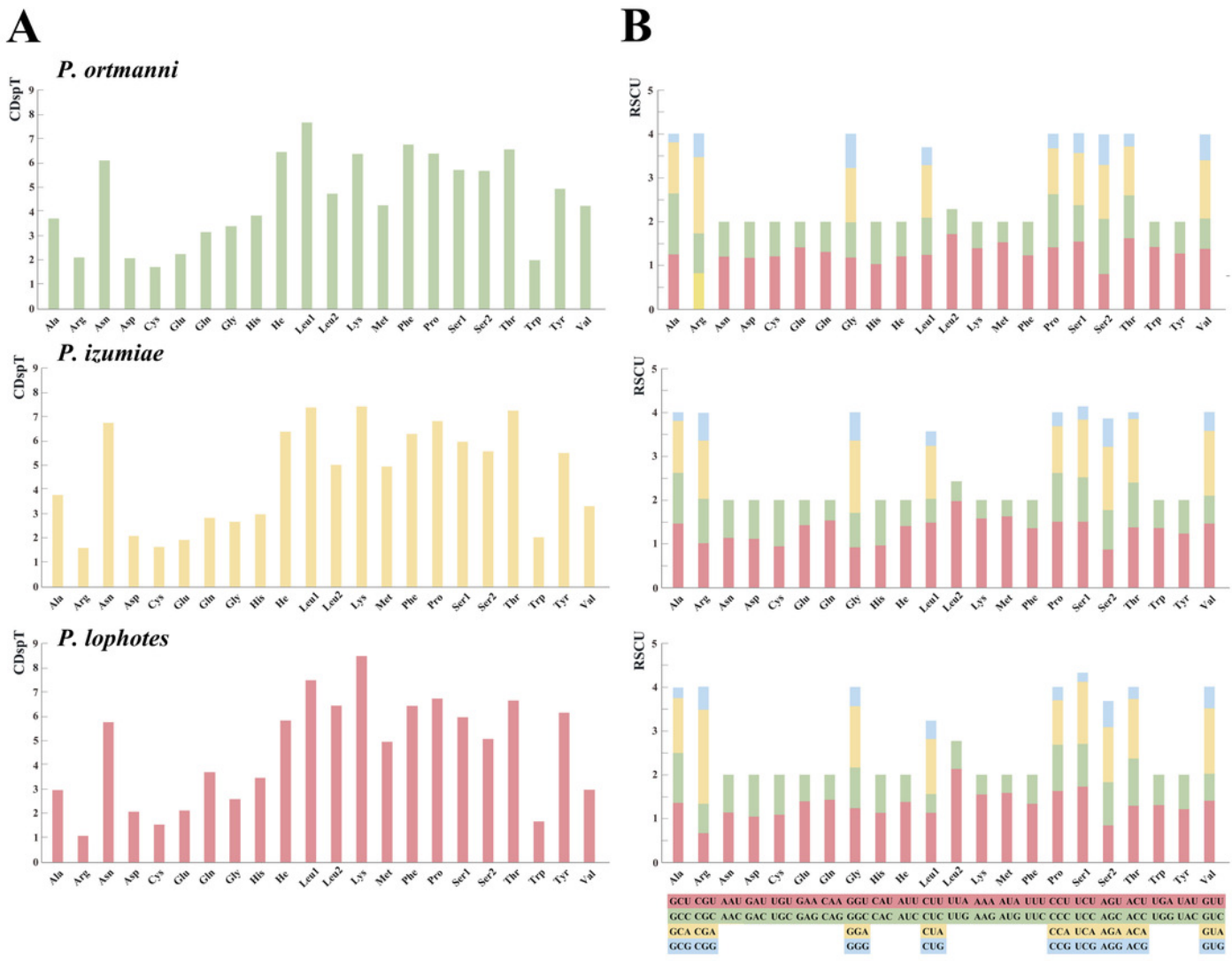


Table 1(on next page)

Sampling locations and dates for the three samples.

Table 1. Sampling locations and dates for the three samples.

Species name	Sampling date	Species location	GenBank
Plesionika ortmanni	April 2022	Zhoushan, Zhejiang Province 122°14′ N, 29°97′ E	OP650932
Plesionika izumiae	April 2022	Zhoushan, Zhejiang Province 122°14′ N, 29°97′ E	OP650933
Plesionika lophotes	April 2021	Taizhou, Zhejiang Province 121°43′ N, 28°68′ E	OP650934

Figure 6

Linear repres entation of the mitochondrial gene arrangement of the ancestral mitogenome of pancrustaceans and Caridea species . In this study, the three newly sequenced species are marked with blue box.

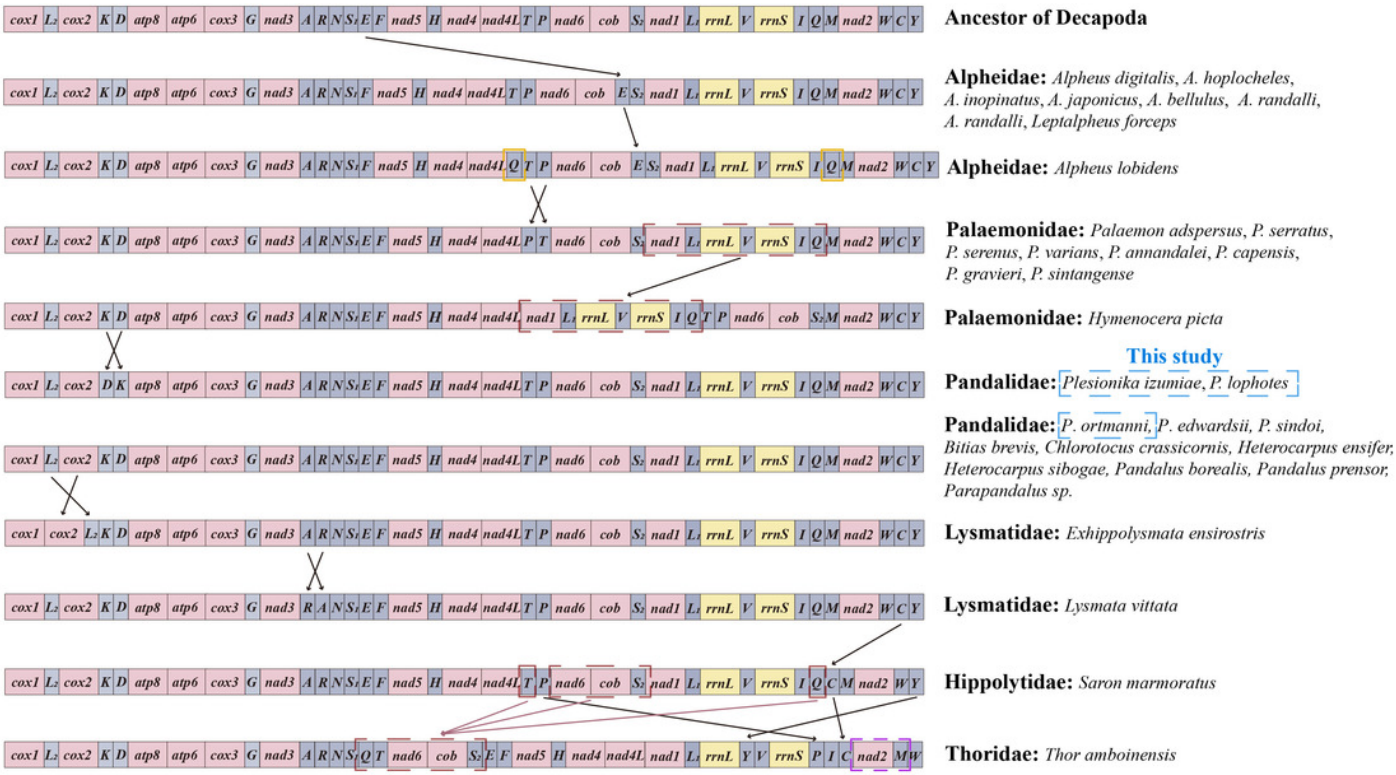
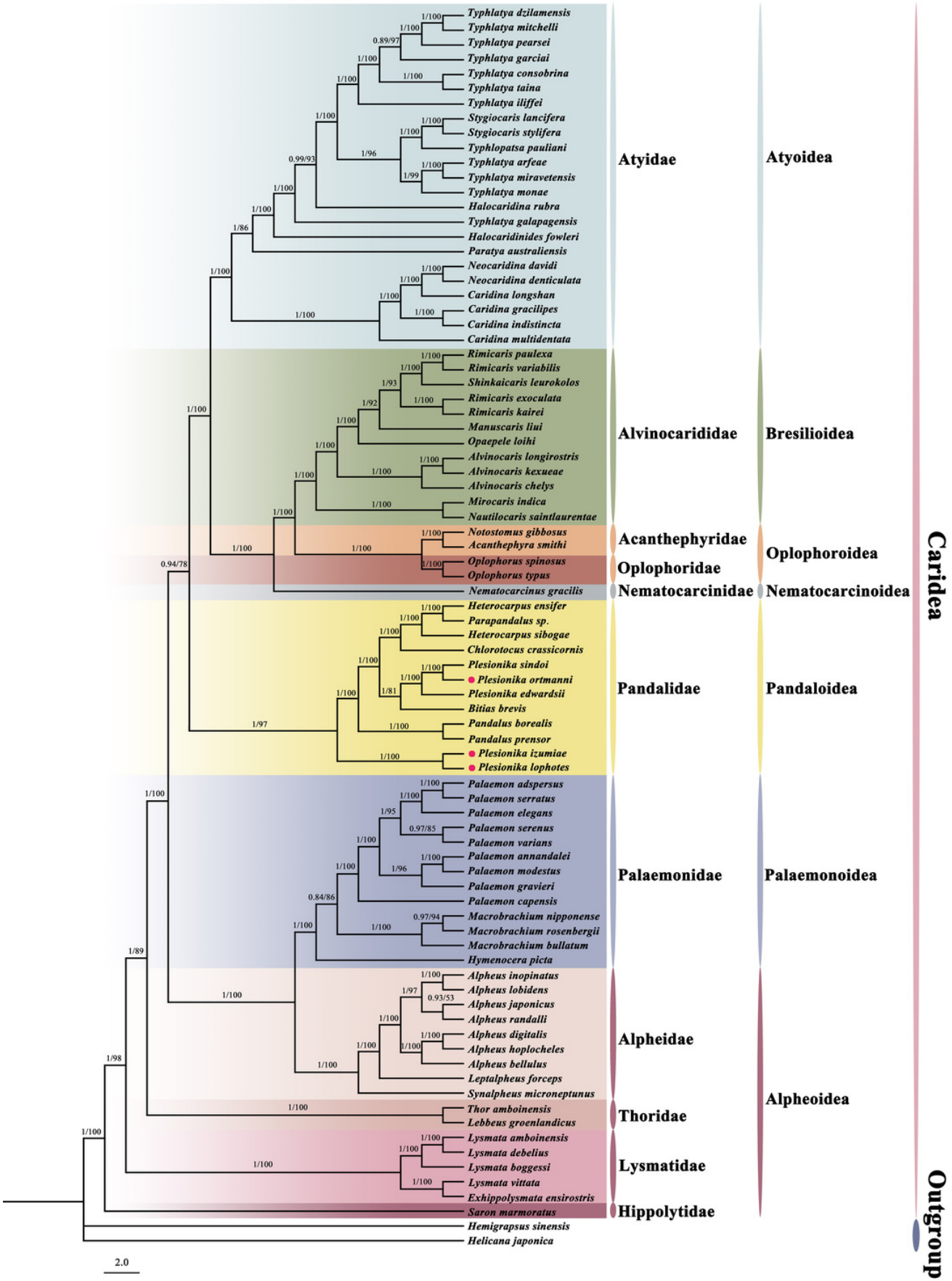


Figure 7

The phylogenetic tree based on 13 PCGs was inferred using Bayesian inference (BI) and maximum likelihood (ML) methods. The number at each clade is the bootstrap probability and the three newly sequenced species are marked with red dots.


Typhlatya dzilamensis
Typhlatya mitchelli
Typhlatya pearsei
Typhlatya garciai
Typhlatya consobrina
Typhlatya taina
Typhlatya iliffei
Stygiocaris lancifera
Stygiocaris stylifera
Typhlopatsa pauliani
Typhlatya arfeae
Typhlatya miravetensis
Typhlatya monae
Halocaridina rubra
Typhlatya galapagensis
Halocaridinides fowleri
Paratya australiensis
Neocaridina davidi
Neocaridina denticulata
Caridina longshan
Caridina gracilipes
Caridina indistincta
Caridina multidentata
Rimicaris paulexa
Rimicaris variabilis
Shinkaicaris leurokolos
Rimicaris exoculata
Rimicaris kairei
Manuscaris liui
Opaepele loihi
Alvinocaris longirostris
Alvinocaris kexueae
Alvinocaris chelys
Mirocaris indica
Nautilocaris saintlaurentae
Notostomus gibbosus
Acanthephyra smithi
Oplophorus spinosus
Oplophorus typus
Nematocarcinus gracilis
Heterocarpus ensifer
Parapandalus sp.
Heterocarpus sibogae
Chlorotocus crassicornis
Plesionika sindoi
Plesionika ortmanni
Plesionika edwardsii
Bitias brevis
Pandalus borealis
Pandalus prensor
Plesionika izumiae
Plesionika lophotes
Palaemon adspersus
Palaemon serratus
Palaemon elegans
Palaemon serenus
Palaemon varians
Palaemon annandalei
Palaemon modestus
Palaemon gravieri
Palaemon capensis
Macrobrachium nipponense
Macrobrachium rosenbergii
Macrobrachium bullatum
Hymenocera picta
Alpheus inopinatus
Alpheus lobidens
Alpheus japonicus
Alpheus randalli
Alpheus digitalis
Alpheus hoplocheles
Alpheus bellulus
Leptalpheus forceps
Synalpheus microneptunus
Thor amboinensis
Lebbeus groenlandicus
Lysmata amboinensis
Lysmata debelius
Lysmata boggessi
Lysmata vittata
Exhippolysmata ensirostris
Saron marmoratus
Hemigrapsus sinensis
Helicana japonica
2.0
Atyidae
Alvinocarididae
Acanthephyridae
Oplophoridae
Nematocarcinidae
Pandalidae
Palaemonidae
Alpheidae
Thoridae
Lysmatidae
Hippolytidae
Atyoidea
Bresilioidea
Oplophoroidea
Nematocarcinoidea
Pandaloidea
Palaemonoidea
Alpheoidea
Caridea
Outgroup