Novel mitochondrial gene rearrangements pattern in the millipede *Polydesmus* sp. GZCS-2019 and phylogenetic analysis of the Myriapoda

Qing Zuo¹ | Zhisheng Zhang¹ | Yanjun Shen²

¹Key Laboratory of Eco-Environments in Three Gorges Reservoir Region (Ministry of Education), School of Life Sciences, Southwest University, Chongqing, China
²Chongqing Key Laboratory of Animal Biology, School of Life Sciences, Chongqing Normal University, Chongqing, China

**Correspondence**
Zhisheng Zhang, Key Laboratory of Eco-Environments in Three Gorges Reservoir Region (Ministry of Education), School of Life Sciences, Southwest University, Chongqing 400715, China.
Email: zhangzs327@qq.com
Yanjun Shen, Chongqing Key Laboratory of Animal Biology, School of Life Sciences, Chongqing Normal University, Chongqing 401331, China.
Email: shenyanjun@cqnu.edu.cn.

**Funding information**
the Science and Technology Research Program of Chongqing Municipal Education Commission, Grant/Award Number: KJQN201900502

**Abstract**
The subphylum Myriapoda included four extant classes (Chilopoda, Symphyla, Diplopoda, and Pauropoda). Due to the limitation of taxon sampling, the phylogenetic relationships within Myriapoda remained contentious, especially for Diplopoda. Herein, we determined the complete mitochondrial genome of *Polydesmus* sp. GZCS-2019 (Myriapoda: Polydesmida) and the mitochondrial genomes are circular molecules of 15,036 bp, with all genes encoded on + strand. The A+T content is 66.1%, making the chain asymmetric, and exhibits negative AT-skew (−0.236). Several genes rearrangements were detected and we propose a new rearrangement model: “TD (N\R) L + C” based on the genome-scale duplication + (non-random/random) loss + recombination. Phylogenetic analyses demonstrated that Chilopoda and Symphyla both were monophyletic group, whereas Pauropoda was embedded in Dipllopoda to form the Dignatha. Divergence time showed the first split of Myriapoda occurred between the Chilopoda and other classes (Wenlock period of Silurian). We combine phylogenetic analysis, divergence time, and gene arrangement to yield valuable insights into the evolutionary history and classification relationship of Myriapoda and these results support a monophyletic Progoneata and the relationship (Chilopoda + (Symphyla + (Diplopoda + Pauropoda))) within myriapod. Our results help to better explain the gene rearrangement events of the invertebrate mitogenome and lay the foundation for further phylogenetic study of Myriapoda.

**KEYWORDS**
evolutionary history, gene rearrangement, Myriapoda

**TAXONOMY CLASSIFICATION**
Evolutionary ecology
1 | INTRODUCTION

Now Myriapoda is looked as a subphylum of Arthropoda, including four classes: Pauropoda, Symphyla, Diplopoda (millipedes), and Chilopoda (centipedes). It is known that Myriapoda first settled in terrestrial ecosystems in the Early Paleozoic, (Lozano-Fernandez et al., 2016), with primitive body shapes, making them play a particularly important role in evolutionary analysis (Dunham, 2012; Giribet & Edgecombe, 2019). Morphological studies hypothesized that the Diplopoda and Pauropoda clustered together to form the Dignatha with the second maxillary segment being limbless in the two pairs of gnathal appendages (Dohle, 1980; Pocock, 1893; Shelley & Golovatch, 2011; Tiegs, 1947). In addition, morphology supported that the Symphyla and Dignatha (Pauropoda + Diplopoda) together formed the taxon Progoneata (Dohle, 1980; Pocock, 1893) based on their common morphological characteristics: the location of the genital opening is near the front of the trunk (Blanke & Wesener, 2014). Chilopoda was presumed to have a sister relationship with the Progoneata (Blanke & Wesener, 2014; Dohle, 1980; Edgecombe, 2006, 2011; Gai et al., 2008; Moritz & Brown, 1987; Read & Enghoff, 2009; Wilson & Anderson, 2004).

In recent decades, with the development of molecular biology, a relatively new field of molecular analysis based on mitochondrial and transcriptome data is flourishing. In contrast to this traditional morphology view, several molecular studies contradicted the Dignatha clade and supported Symphyla + Pauropoda group formed Edafopoda (Andreas et al., 2012; Dong et al., 2012; Fernandez et al., 2018; Gai et al., 2006; Rehm et al., 2014). Therefore, the relationship among the four classes of myriapod is still controversial and the major source of conflict is between molecular and morphological phylogeny.

The millipedes (Diplopoda) is an important component of the modern terrestrial ecosystem due to its important role in the decomposition of organic matter. Hitherto, the Diplopoda contained more than 18,000 species worldwide, which are distributed in most parts of China (Golovatch & Liu, 2020; Jiang & Chen, 2018). Although Diplopoda is the third most diverse class of Myriapoda, there is no widely accepted consensus about the classification and phylogenetic relationship. With the development of molecular biology technology, a new era of phylogenetic analysis of phylogeny has been opened in the early 1990s, and a large number of analyses of millipedes have been published (Brewer et al., 2013; Dong et al., 2016; Lavrov et al., 2002; Liu et al., 2017; Means et al., 2021; Qu et al., 2020; Wesener et al., 2010; Zhao et al., 2020). However, due to the limitation of taxon sampling and the lack of mitochondrial genome data, the previous phylogenetic studies failed to solve the relationship of millipedes.

The mitochondrial genomes of metazoans exhibit variation in many characteristics, such as length, tRNA secondary structure, gene rearrangement, and structure of control regions (Boore, 1999; Mukundan et al., 2020; Shan et al., 2017, 2020). Studying the variation in these characteristics can discover the evolutionary relationship between taxa with a high and/or low classification level. Among them, gene arrangements are relatively complex and diverse, which can become a source of information for system evolution analysis. Furthermore, it also affects the process of mRNA transcription, substitution, and processing. In recent years, the mitochondrial genome rearrangement has been widely studied focusing on phylogenetic relationship and rearrangement mechanism (Feng et al., 2021; Gong et al., 2020; Li et al., 2019, 2020; Powell et al., 2020; Tyagi et al., 2020; Wang et al., 2020; Zhang et al., 2020). In addition, the high rearrangement rate makes the Myriapoda an ideal group to study the interaction between gene rearrangement and phylogenetic relationship. For example, the studies discussed the gene arrangement of Myriapoda on phylogenetic inference but did not prove the universality of this mechanism in the same order species (Gai et al., 2008; Lavrov et al., 2002). Some studies found that the gene arrangement pattern was a sound molecular evidence supporting the Helminthomorpha clade (Brewer et al., 2013; Dong et al., 2012), but they did not elaborate the evolutionary implications of gene arrangements in the Myriapoda. Several common models have been used to explain the gene rearrangement events in the current animal mtDNA, for example: recombination models involved in DNA strand breaks and recombination (Lunt & Hyman, 1997); the Tandem duplication-random loss (TDRL) model is commonly used to support gene tandem replication and random loss (Moritz & Brown, 1987); and the TDNL model supports gene tandem replication and non-random loss (Lavrov et al., 2002). However, the gene rearrangement phenomenon may not be explained by one of the above-mentioned mechanisms alone for some species. Therefore, it is necessary to conduct comparative evolutionary studies on mitogenome rearrangements to accurately identify the mechanisms leading to the rearrangements.

In the present study, we sequenced the complete mitochondrial genome of a millipede, Polydesmus sp. GZCS-2019 (P. GZCS-2019) (Diplopoda: Polydesmidae), and described the genome-scale gene rearrangement events of the mitogenome, providing independent molecular evidence to explore the phylogenetic relationship of Myriapoda. To build a better phylogenetic relationship and understand the evolutionary significance of gene arrangement in Myriapoda, the other 27 complete mitochondrial genomes of the Myriapoda (8 from Chilopoda, 13 from Diplopoda, 2 from Symphyla, and 1 from Pauropoda) and 3 outgroup species were used in this study. Meanwhile, we combine phylogenetic analysis, divergence time, and gene arrangement to yield valuable insights into the evolutionary history and classification relationship of Myriapoda.

2 | MATERIALS AND METHODS

2.1 | Specimen collection and DNA extraction

Two specimens of P. GZCS-2019 were collected from Chishui of Guizhou Province in China (28°24′25″N, 105°57′17″E) in August 2019. Morphological identification of specimens was mainly referred to as “PICTORIAL KEYS TO SOIL ANIMALS OF China” (Yin, 1998) and all specimens were stored in anhydrous ethanol in the Chongqing Key Laboratory of Animal Biology, Chongqing Normal University. Total DNA was extracted from the dehydrated muscle tissues using the TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa Biotech).
2.2 Mitochondrial genome sequencing and assembly

The entire mitogenome of *P. GZCS-2019* was sequenced on the Illumina HiSeq TM platform with paired ends of 300–500 bp. The raw paired reads were quality trimmed using FastQC v0.11.4 (www.bioinformatics.babraham.ac.uk/projects/fastqc/) with default parameters. Finally, yielded 10G raw reads (coverage 3–5x) and clean sequence reads were assembled in the NOVOPlasty (https://github.com/ndierckx/NOVOPlasty) (Nicolas et al., 2016) using sequences from each of the 23 mitochondrial genes of the closest relative available from NCBI as mapping reference, with the default parameter.

2.3 Sequence analysis and gene annotation

The online tool MITOS (http://mitos2.bioinf.uni-leipzig.de/index.py) was used to perform gene annotation, and the annotation results were verified by the BLAST program from the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Donath et al., 2013). Then, the abnormal start codon and stop codon were determined based on the comparison with other millipedes. The relative synonymous codon usage (RSCU) was obtained using MEGA 7.0 (Kumar et al., 2015), which was calculated using PCG with incomplete codons removed. The ribosomal RNA genes were determined according to the location of adjacent tRNA genes and comparison with other Myriapoda mitogenomes from NCBI. The strand asymmetry was calculated using the following formula: AT-skew = (A − T)/(a + T); GC-skew = (G − C)/(G + C) (Perna & Kocher, 1995). The online mitochondrial visualization tool Organellar Genome DRAW (Marc et al., 2013) was used to draw a graphical map of the mitochondrial genome. The secondary cloverleaf structure and the locations of tRNAs were examined with tRNAscan-SE 1.21 (Lowe & Chan, 2016).

2.4 Phylogenetic reconstruction

The mitochondrial genomes used for phylogenetic analysis in this study were all from GenBank, including 24 species of Myriapoda and 3 species of outgroup (1 Decapoda species and 2 Hexapoda species). The species information is shown in Table 1. This phylogenetic analysis is based on 37 genes, including 13 protein coding genes (PCG), 2 ribosomal RNA genes (rRNAs) and 22 transfer RNA genes (tRNAs). The sequences above were aligned by ClustalW method in MEGA 7 (Kumar et al., 2015), with the default parameters. The Gblocks version 0.91b (Castresana, 2000) with the default parameters setting was used for filtering of poorly aligned regions. The aligned sequences of each gene were concatenated using Sequence Matrix v1.7 (Castresana, 2000).

Phylogenetic trees were constructed using the following three datasets: (1) 13 PCGs matrices consisting of 9140 nt; (2) 13 PCG and 2 rRNA matrices composed of 10,884 nt; (3) 13 PCGs, 2 rRNAs, and 10 tRNA matrices composed of 11,459 nt. For these three datasets, the best fitting model GTR + I + G was selected by jModelTest 2 (Darriba et al., 2012) for maximum likelihood (ML) and Bayesian inference (BI) analysis. The ML analysis was assembled in PhyML 3.0 (Stéphane & Olivier, 2003) with fast likelihood-based method and performed 1000 repetitions. Bayesian analyses were carried out using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) under the best-fit models with 10,000,000 generations in two runs of eight chains each and each one was sampled every 200 generations with a burn-in of 25%. Trees inferred prior to stationarity were discarded as burn-in, and those remaining were used to construct a 50% majority rule consensus tree. All phylogenetic trees were viewed and edited using Figtree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree).

2.5 Divergence time estimation

Beast v1.8.4 (Drummond et al., 2012) was used to estimate the divergence time, using the Bayesian analysis method. At the same time, Beauti v1.8.3 was used to generate the beast XML file using uncorrelated lognormal distribution relaxed clock model and the Yule speciation was used to prior process the tree. Two fossil constraints were used in this study: the oldest uncontested terrestrial animal *Pneumodesmus newmani* (421–426 Mya) (Shear et al., 1998) and the oldest terrestrial myriapod body fossil *Rhyniella praecursor* (407–411 Mya) (Wilson & Anderson, 2004). The GTR + I + G model was used to estimate time, and after a burn-in of the initial 50% cycles, divergence times were sampled once every 1000 generations from 100 million Markov Chain Monte Carlo (MCMC) iterations.

The treeAnnotator v1.6.1 (BEAST software) was used to annotate the sampled trees, and the Figtree v1.3.1 was used to conduct the visualization. The ESSs were used to determining the Bayesian statistical significance of each parameter in TRACER v1.5 (ESS > 200) (Rambaut & Drummond, 2003).

3 RESULTS AND DISCUSSION

3.1 Genome structure, organization, and composition

The complete mitogenome sequence of *P. GZCS-2019* is a closed circular molecule with a size of 15,036 bp (Figure 1 and Table 1). In addition, the gene content also conforms to the typical characteristics of other Diplopoda species, including 13 PCGs (cox1-3, nad1-6, nad4L, cob, atp6, and atp8), 2 rRNA genes (rrn5 and rrnL), 22 tRNA genes, and a control region, and all genes are encoded on the heavy (+) chain (Figure 1 and Table 2). Moreover, the mitogenome contains 351 bp intergenic spacer sequences, distributed in 19 regions, ranging in size from 1 to 174 bp (Table 2), and there is a 27 bp overlap between genes in five locations, showing five pairs of overlapping genes: atp8/atp6, rrnS/trnV, trnP/nad4L, nad4L/nad4, and trnH/nad5, of which the longest 7 bp overlap is located between trnL1 and rrnL, nad4L and nad4. Furthermore, the whole mitogenome of
| Species | Taxonomic position | Size (bp) | GenBank no. | Reference |
|---------|--------------------|-----------|-------------|-----------|
| **Polydesmus sp. GZCS-2019** | Diplopoda; Helminthomorpha; Polydesmida; Polydesmidae | 15,036 | MZ677220 | This study |
| **Appalachioria falcifera** | Diplopoda; Helminthomorpha; Polydesmida; Xystodesmidae | 15,282 | JX437063 | Brewer et al. (2013) |
| **Xystodesmus sp. YD-2016** | Diplopoda; Helminthomorpha; Polydesmida; Xystodesmidae | 15,791 | KU721886 | Dong et al. (2016) |
| **Asiomorpha coarctata** | Diplopoda; Helminthomorpha; Polydesmida; Paradoxosomatidae | 15,644 | KU721885 | Dong et al. (2016) |
| **Anaulaciulus koreanus** | Diplopoda; Helminthomorpha; Julida; Julidae | 14,916 | KX096886 | Unpublished |
| **Antrokoreana gracilipes** | Diplopoda; Helminthomorpha; Julida; Nemasomatidae | 14,747 | DQ344025 | Unpublished |
| **Brachycybe lecontii** | Diplopoda; Helminthomorpha; Playtdesmida; Androgнатhidae | 15,115 | JX437064 | Brewer et al. (2013) |
| **Abacion magnun** | Diplopoda; Helminthomorpha; Callipodida; Callipodidae | 15,160 | JX437062 | Brewer et al. (2013) |
| **Thyropygus sp. DVL-2001** | Diplopoda; Helminthomorpha; Spirostreptida; Harpagophoridae | 15,133 | AY055728 | Lavrov et al. (2002) |
| **Narceus annularus** | Diplopoda; Helminthomorpha; Spirobolidae | 14,868 | AY055727 | Lavrov et al. (2002) |
| **Spaerotheriidae sp. HYS-2012** | Diplopoda; Helminthomorpha; Spaerotheriida; Spaerotheriidae | 14,970<sup>a</sup> | JQ713564 | Dong et al. (2012) |
| **Glomeridesmus sp. ITVB918** | Diplopoda; Pentazonia; Glomeridesmida; Glomeridesmidae | 14,848 | MG905160 | Unpublished |
| **Glomeridesmus spelaeus** | Diplopoda; Pentazonia; Glomeridesmida; Glomeridesmidae | 14,819 | MG372113 | Nunes et al. (2020) |
| **Mecistocephalus marmoratus** | Chilopoda; Pleurostigmophora; Geophilomorpha; Mecistocephalidae | 15,279 | KX774322 | Unpublished |
| **Strigamia maritima** | Chilopoda; Pleurostigmophora; Geophilomorpha; Linotaeniidae | 14,983 | KP173664 | Robertson et al. (2015) |
| **Bothropolyx sp. SP-2004** | Chilopoda; Pleurostigmophora; Lithobiomorpha; Ethopolyidae | 15,139 | AY691655 | Unpublished |
| **Cermatobius longicornis** | Chilopoda; Pleurostigmophora; Lithobiomorpha; Henicopidae | 16,833 | KC155628 | Gai et al. (2013) |
| **Lithobius forficatus** | Chilopoda; Pleurostigmophora; Lithobiomorpha; Lithobiidae | 15,695 | AF309492 | Lavrov et al. (2000) |
| **Scolopocryptops sp. 1 YG-2013** | Chilopoda; Pleurostigmophora; Scolopendromorpha; Cryptopidae | 15,119 | KC200076 | Gai et al. (2014) |
| **Scolopendra subspinipes dehaani** | Chilopoda; Pleurostigmophora; Scolopendromorpha; Scolopendridae | 14,538<sup>a</sup> | KY947341 | Unpublished |
| **Scutigera coleoptrata** | Chilopoda; Notostigmophora; Scutigeromorpha; Scutigeridae | 14,922 | AJ507061 | Negrisolo et al. (2004) |
| **Symphyrella sp. YG-2006** | Chilopoda; Notostigmophora; Scutigeromorpha; Scutigeridae | 14,667 | EF576853 | Gai et al. (2008) |
| **Scutigerella causeyae** | Chilopoda; Notostigmophora; Scutigeromorpha; Scutigeridae | 14,637 | DQ666065 | Podsiadlowski et al. (2007) |
| **Pauropus longiramus** | Pauropoda; Pauropodidae | 14,487 | HQ457012 | Dong et al. (2012) |
| **Outgroup** | | | | |
| **Japyx solifugus** | Hexapoda; Japygidae | 15,785 | NC007214 | Carapelli et al. (2005) |
| **Penaeus monodon** | Crustacea; Decapoda; Dendrobranchiata; Penaeidae | 15,984 | AF217843 | Wilson et al. (2000) |
| **Petrobius brevistylis** | Hexapoda; Machilidae | 15,698 | NC007688 | Podsiadlowski (2006) |

Note: Bolded text represents the species in this study.

<sup>a</sup>Incomplete mitogenome.
P. GZCS-2019 is biased toward AT nucleotides (66.1%), similarly to Abacion magnum (66.6%), which belongs to the Callipodida (Table 1). The mitogenome of P. GZCS-2019 has been deposited in NCBI under GenBank accession number MZ677220.

### 3.2 | PCGs and codon usage

In the mitogenome of P. GZCS-2019, the size of the PCGs region is 9882 bp, and all the PCGs genes are encoded on the + strand (Table 2). Furthermore, its mitochondrial DNA is similar to that of other invertebrates, with the eight typical PCGs (nad1, cox1, cox2, cob, cox3, atp8, nad4, and nad6) start with the standard ATG starting codon, nad2 with GTG as the starting codon, nad4L and nad5 with ATC as it, atp6 and nad3 with ATA as it.

Meanwhile, cox1, cox2, and cox3 use TAA as the termination codon; atp8, nad3, and nad4L use TAG as the termination codon; nad5 use TA as the termination codon, while atp6, nad6, cob, nad4L, nad4, and nad2 are terminated by a single T (Table 2). These features are somewhat similar to other invertebrate mitochondrial genomes, and the truncated stop codon may be completed in the form of TAA and TAG through post-transcriptional polyadenylation (Ojala et al., 1981). In the 13 PCGs of P. GZCS-2019, 5012 codons are showed and the most common amino acids are Leu (UUR) (477), Ile (AUR) (295), and Phe (UUR) (462) (Table S1 and Figure S4).

### 3.3 | Skewness, transfer RNAs, and ribosomal RNAs

The nucleotide composition of the mitogenome of P. GZCS-2019 is as follow: A (25.3%), T (40.9%), G (24.2%), and C (9.7%) (Table 3). The whole mitochondrial genome of P. GZCS-2019 exhibits chain asymmetry. The AT-skew of this whole mitochondrial genome is negative (~0.236), indicating that the occurrence of Ts is higher than that of As. At the same time, the GC-skew of this whole
mitochondrial genome is positive (+0.429), indicating that the occurrence of Gs is higher than that of Cs. Similar results were observed in Asiomorpha coarctata, Xystodesmus sp. YD-2016, and Appalachioria falcifera. Ultimately, the nucleotide bias was assessed (Table 3), and millipede from the same order gave similar results, the negative AT-skew and positive GC-skew is a common feature of Polydesmida.

In the mitochondrial genome of P. GZCS-2019, there are 22 tRNAs encoded on the + strand and with a typical cloverleaf structure, which are the common characteristics of the mitogenome of most millipedes. At the same time, the size of these tRNAs was between 57 and 69 bp, showing a strong A+T bias (67.8%) and a slight skew of T versus A (AT-skew = −0.079) (Table 3). The canonical cloverleaf secondary structure is observed in all the other tRNAs.

### Table 2: Features of the mitochondrial genome of Polydesmus sp. GZCS-2019

| Gene | Position no. | Length (bp) | Start codon | Stop codon | Anticodon | Intergenic length | Strand |
|------|--------------|-------------|-------------|------------|-----------|-------------------|--------|
| cox1 | 1-1533       | 1533        | ATG         | TAA        |           | +                 |        |
| cox2 | 1537-2214    | 678         | ATG         | TAA        | +3        | +                 |        |
| tmK  | 2215-2278    | 64          |             |            | AAG       | +                 |        |
| tmD  | 2282-2346    | 65          |             |            | GAC       | +                 | +      |
| atp8 | 2347-2505    | 159         | ATG         | TAG        |           | +                 | +      |
| atp6 | 2502-3165    | 664         | ATA         | T          | −4        | +                 | +      |
| cox3 | 3166-3951    | 786         | ATG         | TAA        |           | +                 | +      |
| tmG  | 3953-4017    | 65          |             |            | GGA       | +1                | +      |
| nad3 | 4025-4369    | 345         | ATG         | TAG        | +7        | +                 | +      |
| tmA  | 4378-4439    | 62          |             |            | GCA       | +8                | +      |
| tmR  | 4440-4505    | 66          |             |            | CGA       | +                 | +      |
| tmN  | 4509-4574    | 66          |             |            | AAC       | +3                | +      |
| tmS1 | 4575-4632    | 58          |             |            | AGC       | +                 | +      |
| tmE  | 4636-4697    | 62          |             |            | GAA       | +3                | +      |
| nad6 | 4698-5166    | 469         | ATG         | T          |           | +                 | +      |
| cob  | 5167-6271    | 1105        | ATG         | T          |           | +                 | +      |
| tmS2 | 6297-6353    | 57          |             |            | TCA       | +25               | +      |
| Control region | 6354-6790  | 437         |             |            |           | +                 | +      |
| rns  | 6791-7598    | 808         |             |            |           | +                 | +      |
| tmV  | 7596-7659    | 64          |             |            | GTA       | −3                | +      |
| rnl  | 7834-8887    | 1054        |             |            |           | +174              | +      |
| tmL1 | 8903-8966    | 64          |             |            | CTA       | +15               | +      |
| tmL2 | 8977-9042    | 66          |             |            | TTA       | +10               | +      |
| nad1 | 9044-9968    | 925/952     | ATG         | T/         | +1        | +                 | +      |
| tmP  | 9969-10,033  | 65          |             |            | CCA       | +                 | +      |
| nad4L| 10,028-10,315| 288         | ATC         | TAG        | −6        | +                 | +      |
| nad4 | 10,309-11,650| 1342        | ATG         | T          | −7        | +                 | +      |
| tmT  | 11,651-11,717| 67          |             |            | ACA       | +                 | +      |
| tmH  | 11,789-11,857| 69          |             |            | CAC       | +71               | +      |
| nad5 | 11,851-13,550| 1700        | ATG         | TA         | −7        | +                 | +      |
| tmF  | 13,559-13,625| 67          |             |            | TTC       | +8                | +      |
| tmY  | 13,626-13,688| 63          |             |            | TAC       | +                 | +      |
| tmQ  | 13,691-13,756| 66          |             |            | CAA       | +2                | +      |
| tmC  | 13,766-13,831| 66          |             |            | TGC       | +9                | +      |
| tmI  | 13,833-13,898| 66          |             |            | ATC       | +1                | +      |
| tmN  | 13,900-13,963| 64          |             |            | ATG       | +1                | +      |
| nad2 | 13,970-14,972| 1003        | GTG         | T          | +6        | +                 | +      |
| tmW  | 14,973-15,035| 63          |             |            | TGA       | +                 | +      |
except trnS1 and trnS2 without dihydrouridine (DHU) arm (Figure S2). In general, such deletion of DHU arm in the secondary structure of trnS1 and trnS2 was considered a common condition in the Diplopoda mitogenome (Brewer et al., 2013; Dong et al., 2016). The stems of cloverleaf secondary include mostly normal base pairs and multiple non-Watson–Crick base pairs. Furthermore, the most

### TABLE 3 Composition and skewness of Polydesmus sp. GZCS-2019 mitogenomes in this study

| Polydesmus sp. GZCS-2019 | Size (bp) | A% | T% | G% | C% | AT (%) | GC (%) | AT-skew | GC-skew |
|--------------------------|----------|----|----|----|----|--------|--------|---------|---------|
| Mitogenome               | 15,036   | 25.3 | 40.9 | 24.2 | 9.7 | 66.1   | 33.9   | -0.236  | 0.429   |
| PCGs                     | 10,997   | 22.5 | 42.3 | 25.1 | 9.9 | 64.9   | 35.1   | -0.305  | 0.430   |
| cox1                     | 1533     | 22.9 | 42.6 | 13.4 | 21.1 | 65.5   | 34.5   | -0.301  | 0.225   |
| cox2                     | 678      | 24.0 | 40.6 | 22.7 | 12.7 | 64.6   | 35.4   | -0.256  | 0.283   |
| cox3                     | 786      | 19.34 | 42.2 | 27.2 | 11.2 | 61.6   | 38.4   | -0.372  | 0.417   |
| nad1                     | 925      | 22.6 | 42.8 | 25.4 | 9.2 | 65.4   | 34.5   | -0.309  | 0.469   |
| nad2                     | 1003     | 23.2 | 44.5 | 7.7 | 22.7 | 67.7   | 32.3   | -0.314  | 0.525   |
| nad3                     | 345      | 19.4 | 42.9 | 6.7 | 31.0 | 62.3   | 37.7   | -0.377  | 0.646   |
| nad4                     | 1342     | 21.4 | 42.2 | 27.3 | 9.2 | 63.6   | 36.4   | -0.327  | 0.496   |
| nad4L                    | 288      | 22.2 | 42.8 | 32.9 | 5.9 | 61.1   | 38.9   | -0.272  | 0.696   |
| nad5                     | 1700     | 23.6 | 42.8 | 25.4 | 8.2 | 66.4   | 33.6   | -0.289  | 0.513   |
| nad6                     | 469      | 24.5 | 41.8 | 26.0 | 7.7 | 66.3   | 33.7   | -0.260  | 0.544   |
| atp6                     | 664      | 22.9 | 42.5 | 23.5 | 11.1 | 65.4   | 34.6   | -0.299  | 0.356   |
| atp8                     | 159      | 28.3 | 39.6 | 27.0 | 5.0 | 67.9   | 32.1   | -0.167  | 0.686   |
| cob                      | 1105     | 21.7 | 41.7 | 24.1 | 12.5 | 63.4   | 36.6   | -0.315  | 0.317   |
| tRNAs                    | 1415     | 31.2 | 36.6 | 22.0 | 10.1 | 67.8   | 32.2   | -0.079  | 0.371   |
| rRNAs                    | 1862     | 33.1 | 37.1 | 20.8 | 8.97 | 70.2   | 29.8   | -0.056  | 0.397   |
| Control region           | 437      | 34.6 | 38.4 | 21.9 | 5.0 | 72.9   | 27.0   | -0.053  | 0.627   |

Note: Bolded text represents the species in this study.
common non-Watson–Crick base pair is G–U (or U–G) wobble base pairs, which have been known to provide comparable thermodynamic stability to Watson–Crick base pairs and are nearly isomorph to them. The G–U (or U–G) base pairs appear in all 22 tRNAs.

The rrnS (1054 bp) gene located between Control region and trnV, and the rrnL (808 bp) gene located between trnV and trnL1 are encoded on + strand (Table 1 and Figure 1). The A+T content (70.2%) of the rRNA genes is higher than the whole genome (66.1%) (Table 3), with a negative AT-skew ~0.056 (Table 2) and whose structural diagram is shown in Figure S3.

3.4 | Control regions

The largest non-coding region of the mitochondrial genome is usually presumed as the control region and is heavily biased toward A+T nucleotides. The four Polydesmida species mitogenome: P. GZCS-2019, X. YD-2016, A. coarctata, and A. falcifera are compared with the ancestor Limulus polyphemus. We found the non-coding regions of Polydesmida vary in number, size, and location due to the duplications and rearrangement of genome (Figure 2). Our analyses suggested that non-coding region, which is heavily biased to A+T nucleotides, located between trnS2 and rrnS was a putative control region. Besides, there are some common conserved motifs observed in the four Polydesmida species (Figure S7), including the hairpin loop structures, TA(A)n-like stretch, TATA motif, and G(A)nT motif, which were identified as initiation sites for replication and transcription (Boore, 1999; Cameron et al., 2007; Jeffrey, 1999; Shadel & Clayton, 1997; Taannan, 1999; Wei et al., 2013). However, the poly A-stretches at the 5′ and 3′ end of the ancestor L. polyphemus are not observed in the other three Polydesmida species (Figure S6). Therefore, we speculate that this event is responsible for the reverse of strand transcription direction observed in this Polydesmida order and further experiments are needed to clarify this speculation.

3.5 | Gene rearrangement

Compared with ancestral Arthropoda (e.g., L. polyphemus), seven genes and gene blocks (trnF-nad5-trnH-nad4-nad4L-trnP-trnP, nad1-trnL2-trnL1-rrnL-trnV-rrnS, trnT, trnC, trnY, trnL, and trnQ), and AT-rich region (putative control region; CR) have been rearranged in P. GZCS-2019 (Figure 2a). The mitogenome of P. GZCS-2019 is unique compared to other myriapod species; all coding regions are on a single strand. At present, several mature mechanisms have been commonly used to explain gene rearrangement in animal mitogenomes, including duplication-random loss (TDRDL) (Moritz & Brown, 1987), duplication-nonrandom loss (TDNL) (Lavrov et al., 2002), and recombination (Lunt & Hyman, 1997). However, several unique features of P. GZCS-2019 rearrangements prevent the application of these models to this species.

Here, we propose a new rearrangement model: “TD (N/R) L + RC” model based on a genome-scale duplication + (non-random/random) loss + recombination account for the mitogenome gene rearrangement of the P. GZCS-2019. In deducing the rearrangement mechanism of this mito genome, with reference to the theory of the non-random loss (TDNL) model (Lavrov et al., 2002) all but minor rearrangements were found: the trnT translocation and the trnL-trnQ translocation. The first step is the tandem duplication of the entire mitogenome, resulting in a dimeric molecule with two identical monomers covalently linked head to tail (Figure 2b1). Consecutive copies were then followed by a non-random loss of the duplicated genes and the loss of genes would be predetermined by their transcriptional polarity. All genes having one polarity would be lost from one genome copy, and all genes having the opposite polarity would be lost from the other, ending with monomer 1 (trnL1, trnQ, trnM, nad2, trnW, trnC, trnY, cox1, cox2, trnK, trnD, atp8, atp6, cox3, trnG, nad3, trnA, trnR, trnN, trnS1, trnE, trnF, nad5, trnH, nad4, nad4L, trnT, trnP, nad6, cob, trnS2, nad4L, trnL1, rrnL, trnV, rrnS, and CR) and monomer 2 (CR, rrnS, trnV, rrnL, trnL1, trnL2, nad4L, trnS2, cob, nad6, trnP, trnT, nad4L, nad4, trnH, nad3, trnF, trnE, trnS1, trnN, trnR, trnA, nad3, trnG, cox3, atp6, atp8, trnD, trnK, cox1, cox2, trnC, trnW, nad2, trnP, nad4, trnQ, and trnL) (underline denotes the deleted gene; the bold ones are genes that are encoded in the + strand; and the regular ones are genes that are encoded in the − strand) (Figure 2b1). Different from the TDNL model (Lavrov et al., 2002), the 3′ end of monomer 1 is linked to the 3′ end of monomer 2 to form the ultimate gene arrangement of the P. GZCS-2019 mitogenome: (trnL1, trnM, nad2, trnW, cox1, cox2, trnK, trnD, atp8, atp6, cox3, trnG, nad3, trnA, trnR, trnN, trnS1, trnE, nad6, cob, trnS2, CR, rrnS, trnL1, trnL2, nad4L, trnP, nad4L, nad4, trnT, trnH, nad5, trnF, trnY, trnQ, trnC) (the bold ones are genes that are encoded in the + strand; the regular ones are genes that are encoded in the − strand) and the transcription polarity of these genes encoding on the negative strand is reversed, which was shown in Figure 2c. It may be that the non-coding sequences determined and the predicted possible secondary structures play some roles in the early stages of the replication and transcription process (Lavrov et al., 2002; Parker et al., 2009; Tomita et al., 2002). However, further experiments are needed to clarify this speculation.

Tandem duplication-random loss mechanism was widely used to explain the translocation of mitochondrial genes, the trnT translocation phenomenon in this study can be explained by this theory, that occurring in the region between nad4L and trnP, followed by deletions of redundant genes resulting in trnT-nad4-nad4L (Figure 2b2). In contrast, the inversion of trnC-trnQ referred to the transversion of trnL-trnQ, which was more in line with the recombination model (Lunt & Hyman, 1997) (Figure 2b3).

Different from TDNL and TDRDL, the TD (N/R) L + RC model has the following characteristics: the whole genome duplicated and the gene loss according to their transcriptional polarity but not randomly as in the TDRDL; the second is the change in transcription direction and polarity around the control region, which is different from the TDNL method. Indeed, each step of the TD(N/R) L+RC model does not violate the nature and rules of mitochondrial replication. Nevertheless, our presumed model still needs more experimental evidence to verify.
We compared the gene order of *P. GZCS-2019* mitogenome with another three Polydesmida species (*A. coarctata*, *X. YD-2016*, and *A. falcifera*). A striking finding is that all of them were almost arranged in the same way and all coding regions were on a single strand (Figure S5), indicating that this may be a common feature of Polydesmida, and showing that *P. GZCS-2019* had a close evolutionary connection with *A. coarctata*, *X. YD-2016*, and *A. falcifera*. Furthermore, we also found the inversion of the entire side of a genome (trnF-nad5-trnH-nad4-nad4L, trnP, nad1-trnL2-trnL1-rrnS-CR, trnQ, trnC, and trnY) and the translocation of trnT and the inversion of trnC-trnQ could be proposed as common events about gene order in Polydesmida lineage (Figure S5). The duplication-nonrandom loss was also detected in the Symphyla species (Gai et al., 2006, 2008), which reinforce the sister relationship with Diplopoda. These results of the regular gene arrangement in Myriapoda provide useful information for the phylogenetic inference of advanced groups.

### 3.6 | Phylogenetic reconstruction

The concatenated set of nucleotide sequences of the 13 PCGs from 13 Diplopoda species, 8 Chilopoda species, 2 Symphyla species, 1 Pauropoda species, and 3 outgroup species are used for reconstructing phylogenetic relationships among the millipedes by BI and ML methods (Figures 3 and 4). In this study, conserved blocks of amino acid and nucleotide data sets were used to perform the Bayesian inference and maximum likelihood phylogenetic analysis. Phylogenetic analyses based on three datasets matrix demonstrated the relationships of Myriapoda. Both the BI and ML trees support a sister group relationship of Diplopoda + Pauropoda (named Dignatha), which contradicts the Symphyla + Pauropoda group (named Edafopoda) (Figures 3 and 4). The Dignatha group was inferred from morphological data, which shares modified mouthparts, due to the lack of appendage buds on the second maxillary segment (Blanke & Wesener, 2014; Liu et al., 2017; Pocock, 1893). Symphyla is speculated from

![Phylogenetic tree of Polydesmus sp. GZCS-2019](image_url)
the BI and ML trees as a sister group of Dignatha (Figures 3 and 4), traditional morphology classifies it with Dignatha as Progoneata (Diplopoda + Pauropoda + Symphyla) based on their common morphological characteristics: the location of the genital opening is near the front of the trunk (Dohle, 1980; Edgecombe, 2011; Gai et al., 2008; Pocock, 1893; Sierwald & Bond, 2007; Verhoeff, 1913). Meanwhile, the BI and ML analyses showed the basal position of Chilopoda and the interordinal relationships within the Chilopoda (((Lithobiomorpha + Geophilomorpha) Scolopendromorpha) Scutigeromorpha) (Figures 3 and 4, Figure S1B), which was inconsistent with the previous morphological and molecular studies (Bonato et al., 2015; Edgecombe, 2006; Negrisolo et al., 2004).

The Diplopoda was the most numerous species in this study and the extant Diplopoda is divided into two groups: Chilognatha and Penicillata (Blanke & Wesener, 2014; Dohle, 1980; Jiang & Chen, 2018). The Penicillata includes Polyxenida with no species in our analyses (Figures 3 and 4). The Chilognatha includes most of the species in the Diplopoda, which is composed of two monophyletic infraclass Pentazonia and Helminthomorpha (Figure 3, BPP = 1/1/1). The infraclass of Pentazonia is further classified into three orders: Glomerida, Sphaerotheriida, and Glomeridesmida (Figure S1A), contrary to the standard morphological hypothesis that combines Glomerida and Sphaerotheriida into a single clade called Oniscomorpha (Blanke & Wesener, 2014; Iniesta & Wesener, 2012; Sierwald & Bond, 2007). The infraclass Helminthomorpha is composed of two subclasses: Colobognatha and Eugnatha (Figure S1A). Some previous studies support the monophyly of the Helminthomorpha (Brewer et al., 2013; Dong et al., 2016; Pitz & Sierwald, 2010), however, our phylogenetic trees could not support the monophyly of Helminthomorpha, Colobognatha, and Eugnatha because of the incorporation of the Pauropoda (Pauroopus longiramus).

Nonetheless, the BI analyses in this study strongly supported the sister relationship of Pentazonia and Helminthomorpha (Figure 3 and Figure S1A, BPP = 1/1/1). The morphology studies showed the subclass Eugnatha is composed of two sister superorders: Juliformia and Polydesmida (Blanke & Wesener, 2014; Jiang & Chen, 2018; Minelli, 2011), which was strongly supported by our results (Figure 3 and Figure S1A, BPP = 1/1/1). Simultaneously, a sister relationship among Julida, Spirostreptida, and Spirobolida was also strongly supported within the superorder Juliformia (Figure 3 and Figure S1A, BPP = 1/1/1), which is consistent with classical taxonomy (Dohle, 1980; Enghoff et al., 1993; Forrey & Thomas, 1998; Pocock, 1893). Additionally, we found that P. GZCS-2019, A. coarctata, X. YD-2016, and A. falcifera are clustered in one branch with high support value (Figures 3 and 4, Figure S1A, BPP = 1/1/1, ML = 100/100/100). This phenomenon is also supported by the mitochondrial gene rearrangement model deduced above. Although mitochondrial genome gene rearrangement may provide more phylogenetic markers in this study, the analysis of Myriapoda gene rearrangement pattern cannot fully explain the problem of phylogeny, and may have a certain bias.
3.7 Divergence time

Understanding the origin and evolutionary history of myriapods is crucial for interpreting the colonization and evolution of arthropods on land. Hitherto, Myriapoda is inferred to have colonized land in the Early Cambrian, substantially predating body or trace fossil evidence (Giribet & Edgecombe, 2019; Lozano-Fernandez et al., 2016; Wilson & Anderson, 2004). In this study, the Bayesian divergence times showed that the splitting of the ancestral lineages of the Progoneata and Chilopoda from a common ancestor occurred during the Wenlock period of Silurian, slightly earlier than the oldest milipede and centipede fossil records in the Silurian, which was similar to many previous studies (Rosa et al., 2016; Wilson & Anderson, 2004), suggesting that both had experienced the same period of relative stasis period as the plants prior to this (Giribet & Edgecombe, 2019; Lozano-Fernandez et al., 2016; Minter et al., 2017). Then, the Progoneata clade split into Dignatha (Diplopoda + Pauropoda) and Symphyla during the early Silurian to lower Devonian. The results showed that the first split of myriapod occurred between the subclass Chilopoda and other subclasses, rather than between Symphyla and other subclasses, which align with the morphology classification results that supports the monophyly of Progoneata (Figure 5).

In Chilopoda, the split time of Pleurostigmophora and Notostigmophora was from the early Silurian to middle Triassic, slightly earlier than the oldest fossil chilopods in the Late Silurian (Wilson & Anderson, 2004), which was consistent with some previous studies and these were representatives of the Chilopoda (Bonato et al., 2015; Chipman et al., 2014; Giribet & Ed Gecombe, 2013). Moreover, this study also concluded that the split time of Scolopendromorpha and (Lithobiomorpha + Geophilomorpha) was from middle Devonian to early Jurassic and the divergence time of Lithobiomorpha and Geophilomorpha was from early Carboniferous to early Cretaceous (Figure 5).

In Diplopoda, the divergence time of the two infraclasses, Pentazonia and Helminthomorpha, was in the late Silurian to Pennsylvania Carboniferous, during the infraclasse Helminthomorpha. During the subclass Eugnatha, the divergence time between the superorders Juliformia and Polydesmida was from early Devonian to middle Permian. Within the infraclasse Pentazonia, the divergence time between the superorders Glomeridesmida and Sphaerotheriida was from the late Devonian to the late Jurassic period (Figure 5).

4 CONCLUSION

In this paper, we report the complete mitogenome of P. GZCS-2019 (Diplopoda: Polydesmidae) with a novel genome-scale rearrangement phenomenon. We deduce the genome-scale
“duplication + (non-random/random) loss + recombination (TD (N|R) L + RC)” model resulted in a novel mechanism of gene rearrangement for the published Polydesmida mitogenome. The deletion of the DHU arm of trnS1 and trnS2 was considered a common condition in the Polydesmida mitogenome. The phylogenetic analysis supported the monophyletic of Diplopoda, providing evidence for the higher-level relationships within it. Meanwhile, we combine phylogenetic analysis and divergence time to yield valuable insights into the evolutionary history and classification relationship of Myriapoda and these results support a monophyletic Progoneata and the relationship (Chiropoda + (Symphylla + (Diplopoda + Pauropoda))).

Since the mitochondrial gene rearrangement events in Myriapoda contain genetic information related to the phylogenetic evolution of species, it is necessary to conduct in-depth research and use the genetic information revealed by gene rearrangement to better solve these controversial phylogenetic problems. However, due to the lack of taxon samples, there are still many limitations in this study. Therefore, it is necessary to collect more species of Myriapoda for more in-depth and systematic research.

ACKNOWLEDGMENTS
The authors sincerely thank all the crews for their help with the manuscript writing and data analysis. This work was supported by the Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No. KJQN201900502).

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
Qing Zuo: Resources (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). Zhisheng Zhang: Conceptualization (equal); Data curation (equal); Investigation (equal). Yanjun Shen: Formal analysis (equal); Funding acquisition (equal); Investigation (equal).

DATA AVAILABILITY STATEMENT
DNA sequences: GenBank (Accession Numbers MZ677220).

ORCID
Qing Zuo https://orcid.org/0000-0003-4182-6012

REFERENCES
Andreas, Z., Regier, J. C., Zwickl, D. J., & Gadagkar, S. R. (2012). Resolving discrepancy between nucleotides and amino acids in deep-level arthropod phylogenomics: Differentiating serine codons in 21-amino-acid models. PLoS One, 7, e47450.
Bernt, M., Donath, A., Jüthing, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M., & Stadler, P. F. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics & Evolution, 69, 313–319.
Blanke, A., & Wesener, T. (2014). Revival of forgotten characters and modern imaging techniques help to produce a robust phylogeny of the Diplopoda (Arthropoda, Myriapoda). Arthropod Structure & Development, 43, 63–75. https://doi.org/10.1016/j.asd.2013.10.003
Bonato, L., Drago, L., & Muriene, J. (2015). Phylogeny of Geophilomorpha (Chiropoda) inferred from new morphological and molecular evidence. Cladistics – The International Journal of the Willi Hennig Society, 30, 485–507.
Boore, J. L. (1999). Animal mitochondrial genomes. Nucleic Acids Research, 27, 1767–1780. https://doi.org/10.1093/nar/27.8.1767
Brewer, M. S., Swaﬀord, L., Spruill, C. L., & Bond, J. E. (2013). Arthropod phylogenetics in light of three novel millipede (Myriapoda: Diplopoda) mitochondrial genomes with comments on the appropriateness of mitochondrial genome sequence data for inferring deep level relationships. PLoS One, 8, e68005. https://doi.org/10.1371/journal.pone.0068005
Cameron, S. L., Johnson, K. P., & Whiting, M. F. (2007). The mitochondrial genome of the screamer louse Bothriometopus (Phthiraptera: Ischnocera): Effects of extensive gene rearrangements on the evolution of the genome. Journal of Molecular Evolution, 65, 589–604. https://doi.org/10.1007/s00239-007-9042-8
Carapelli, A., Nardi, F., Dallai, R., Boore, J. L., Liò, P., & Frati, F. (2005). Relationships between hexapods and crustaceans based on four mitochondrial genes. Crustacean Issues, 16, 295.
Castrigiana, J. (2000). GBIOCLKS: Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Version 0.91b. Molecular Biology & Evolution, 17, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334
Chipman, A. D., Ferrier, D. E. K., Brena, C., Qu, J., Hughes, D. S. T., Schröder, R., Torres-Oliva, M., Znassi, N., Jiang, H., Almeida, F. C., Alonso, C. R., Apostolou, Z., Aqrawi, P., Arthur, W., Barna, J. C. J., Blankenburg, K. P., Brites, D., Capella-Gutiérrez, S., Coyle, M., … Richards, S. (2014). The first myriapod genome sequence reveals conservative arthropod gene content and genome organisation in the centipede Strigamia maritima. PLoS Biology, 12, e1002005. https://doi.org/10.1371/journal.pbio.1002005
Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. Nature Methods, 9, 772.
Dohle, W. (1980). Sind die Myriapoden eine monophyletische Gruppe? Eine Diskussion der Verwandtschaftsbeziehungen der Antennaten. Abhandlungen des Naturwissenschaftlichen Vereins in Hamburg, 23, 45–103.
Dong, Y., Sun, H., Guo, H., Pan, D., Qian, C., Hao, S., & Zhou, K. (2012). The complete mitochondrial genome of Pauropus longiramus (Myriapoda: Pauropoda): Implications on early diversification of the myriapods revealed from comparative analysis. Gene, 505, 57–65. https://doi.org/10.1016/j.gene.2012.05.049
Dong, Y., Zhu, L., Bai, Y., Ou, Y., & Wang, C. (2016). Complete mitochondrial genomes of two flat-backed millipedes by next-generation sequencing (Diplopoda, Polydesmidia). ZooKeys, 637, 1–20. https://doi.org/10.3897/zookeys.637.9909
Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution, 29, 1185–1192.
Dunham, J. (2012). Encyclopedia of biodiversity. Library Journal, 137, 100.
Edgecombe, G. G. D. (2006). Conflict between datasets and phylogeny of centipedes: An analysis based on seven genes and morphology. Proceedings Biological Sciences, 273, 531–538.
Edgecombe, G. G. D. (2011). 1 Phylogenetic relationships of Myriapoda. In A. Minelli (Ed.), Treatise on zoology-anatomy, taxonomy, biology. The Myriapoda (Vol. 1, pp. 1–20). Brill.
Enghoff, H., Dohle, W., & Blower, J. G. (1993). Anamorphosis in millipedes (Diplopoda)—the present state of knowledge with some developmental and phylogenetic considerations. Zoological Journal
Lavrov, D. V., Boore, J. L., & Brown, W. M. (2002). Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: Duplication and nonrandom loss. Molecular Biology and Evolution, 19, 163-169. https://doi.org/10.1093/oxfordjournals.molehr.a004068

Li, Q., Ren, Y., Shi, X., Peng, L., Zhao, J., Song, Y., & Zhao, G. (2019). Comparative mitochondrial genome analysis of two ectomyorrhizal fungi (Rhizopogon) reveals dynamic changes of intron and phylogenetic relationships of the subphylum Agaricomycotina. International Journal of Molecular Sciences, 20, 5167. https://doi.org/10.3390/ijms20205167

Li, R., Zhang, W., Ma, Z. X., & Zhou, C. F. (2020). Novel gene rearrangement pattern in the mitochondrial genomes of Torleya mikhalli and Cincticostella fusca (Ephemeroptera: Ephemerellidae). International Journal of Biological Macromolecules, 165, 3106–3114. https://doi.org/10.1016/j.ijbiomac.2020.10.124

Liu, W., Wesener, T., Golovatch, S., & Tian, M. (2017). Contributions to the millipede genus Nepalela Shear, 1979 from China, with four new species and first results on phylogeny based on DNA-barcoding (Diplopoda, Chordeumatida, Megalotylidae). Zootaxa, 4243, 455. https://doi.org/10.11646/zootaxa.4243.3.3

Lowe, T. M., & Chan, P. P. (2016). TrNAscan-SE on-line: Integrating search and context for analysis of transfer RNA genes. Nucleic Acids Research, 44, W54–W57.

Lozano-Fernandez, J., Carton, R., Tanner, A. R., Puttick, M. N., Blaxter, M., Vintner, J., Olesen, J., Giribet, G., Edgecombe, G. D., & Pisani, D. (2016). A molecular palaeobiological exploration of arthropod terrestrialization. Philosophical Transactions of the Royal Society B: Biological Sciences, 371, 20150133. https://doi.org/10.1098/rstb.2015.0133

Lunt, D. H., & Hyman, B. C. (1997). Animal mitochondrial DNA recombination. Nature, 387, 247. https://doi.org/10.1038/387247a

Marc, L., Oliver, D., Sabine, K., & Ralph, B. (2013). OrganellarGenomeDRAW—A suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucleic Acids Research, 41, W575–W581

Means, J. C., Hennen, D. A., Tsutomu, T., & Marek, P. E. (2021). Phylogenetic systematics of the millipede family Xystodesmidae. Insect Systematics and Diversity, 5, 1–26. https://doi.org/10.1093/isd/ixab003

Minelli, A. (2011). Treatise on zoology – Anatomy, taxonomy, biology. The Myriapoda (Vol. 1, pp. 230–268). Brill. https://doi.org/10.11646/zootaxa.3550.1.2

Mortiz, C., & Brown, W. M. (1987). Tandem duplications in animal mitochondrial DNAs: Variation in incidence and gene content among lizards. Proceedings of the National Academy of Sciences of the United States of America, 84, 7183–7187. https://doi.org/10.1073/pnas.84.20.7183

Mukundan, L. P., Sukumar, S., Sebastian, W., & Gopalakrishnan, A. (2020). Characterization of the whole mitogenome of largehead hairtail Trichiurus lepturus (Trichiuridae): Insights into special characteristics. Biochemical Genetics, 58, 430–451. https://doi.org/10.1007/s10528-020-09956-z

Minter, N. J., Butoois, L. A., Mángano, M. G., Davies, N. S., Giibling, M. R., MaNaughton, R. B., & Labandeira, C. C. (2017). Early bursts of diversification defined the faunal colonization of land. Nature Ecology & Evolution, 1(7), 1–10.

Negrisolo, E., Minelli, A., & Vallee, G. (2004). Extensive gene order rearrangement in the mitochondrial genome of the centipede Scutigera coleoptrata. Journal of Molecular Evolution, 58, 413–423. https://doi.org/10.1007/s00239-003-2563-x

Nicolas, D., Patrick, M., & Guillaume, S. (2016). NOVOPlasty: De novo assembly of organelle genomes from whole genome data. Nucleic Acids Research, 45, e18.
Perna, N. T., & Kocher, T. D. (1995). Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Molecular Biology and Evolution*, 12(4), 696–704. https://doi.org/10.1093/10635150390235520

Taaman, J. W. (1999). The mitochondrial genome: Structure, transcription, translation and replication. *Biochimica et Biophysica Acta*, 1410, 103–123. https://doi.org/10.1016/S0005-2728(98)00161-3

Tieg, O. W. (1947). The development and affinities of the Pauropoda, based on a study of *Pauropus silvicus*. *Journal of Cell Science*, 88, 275–336.

Tomita, K., Yokobori, S. I., Oshima, T., Ueda, T., & Watanabe, K. (2002). The Cephalopod Loligo bleekeri mitochondrial genome: Multiplied noncoding regions and transposition of tRNA genes. *Journal of Molecular Evolution*, 54, 486–500. https://doi.org/10.1007/s00239-001-0039-4

Tyagi, K., Chakraborty, R., Cameron, S. L., Sweet, A. D., Chandra, K., & Kumar, V. (2020). Rearrangement and evolution of mitochondrial genomes in Thysanoptera (Insecta). *Scientific Reports*, 10, 695. https://doi.org/10.1038/s41598-020-57705-4

Verhoeff, K. W. (1913). Die Ordnungen der Proterandria und zur Kenntnis der Cambaliden (ber Diplopoden. 65. Aufsatz). *Anzeiger für Paläontologie*, 40, 1193. https://doi.org/10.1016/j.ympev.2010.08.023

Wilson, K., Caihii, V., Ballment, E., & Benzie, J. (2000). The complete mitochondrial genome of the crustacean Penaeus monodon: Are malacostracan crustaceans more closely related to insects than to branchiopods? *Molecular Biology and Evolution*, 17(6), 863–874.

Yin, W. (1998). Pictorial keys to soil animals of China. Science press.

Zuo, et al. (2020). D. Perspectives on taxon origins and ages, and a hypothesis on the origin and early evolution of the class. *Center for Systematic Entomology*, 158, 1–134.

Shen, Y., Kou, Q., Zhong, Z., Li, X., He, L., He, S., & Gan, X. (2017). The first complete mitogenome of the South China deep-sea giant isopod Bathyomus sp (Crustacea: Isopoda: Cirolanidae) allows insights into the early mitogenomic evolution of isopods. *Ecology and Evolution*, 7. 1869–1881.

Shen, Y., Yang, N., Liu, Z., Chen, Q., & Li, Y. (2020). Phylogenetic perspective on the relationships and evolutionary history of the Acipenseriformes. *Genomics*, 112, 3511–3517. https://doi.org/10.1016/j.ygeno.2020.02.017

Sierwald, P., & Bond, J. E. (2007). Current status of the myriapod class Diplopoda (Millipedes): Taxonomic diversity and phylogeny. *Annual Review of Entomology*, 52, 401–420. https://doi.org/10.1146/annurev.ente.52.111805.090210
Zhao, Y., Yu, J., & Liu, W. (2020). A molecular-based phylogeny of the millipede genus *Sphaerobelum* Verhoeff, 1924, with the first record of the genus from mainland China (Diplopoda: Sphaerotheriida: Zephroniidae). *Annales Societe Entomologique de France*, 56, 1–8.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

---

**How to cite this article:** Zuo, Q., Zhang, Z., & Shen, Y. (2022). Novel mitochondrial gene rearrangements pattern in the millipede *Polydesmus* sp. GZCS-2019 and phylogenetic analysis of the Myriapoda. *Ecology and Evolution*, 12, e8764. https://doi.org/10.1002/ece3.8764