A Rearrangement of the Mitochondrial Genes of Centipedes (Arthropoda, Myriapoda) with a Phylogenetic Analysis

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Abstract: Due to the limitations of taxon sampling and differences in results from the available data, the phylogenetic relationships of the Myriapoda remain contentious. Therefore, we try to reconstruct and analyze the phylogenetic relationships within the Myriapoda by examining mitochondrial genomes (the mitogenome). In this study, typical circular mitogenomes of *Mecistocephalus marmoratus* and *Scolopendra subspinipes* were sequenced by Sanger sequencing; they were 15,279 bp and 14,637 bp in length, respectively, and a control region and 37 typical mitochondrial genes were annotated in the sequences. The results showed that all 13 PCGs started with ATN codons and ended with TAR codons or a single T; what is interesting is that the gene orders of *M. marmoratus* have been extensively rearranged compared with most Myriapoda. Thus, we propose a simple duplication/loss model to explain the extensively rearranged genes of *M. marmoratus*, hoping to provide insights into mitogenome rearrangement events in Myriapoda. In addition, our mitogenomic phylogenetic analyses showed that the main myriapod groups are monophyletic and supported the combination of the Pauropoda and Diplopoda to form the Dignatha. Within the Chilopoda, we suggest that Scutigeromorpha is a sister group to the Lithobiomorpha, Geophilomorpha, and Scolopendromorpha. We also identified a close relationship between the Lithobiomorpha and Geophilomorpha. The results also indicate that the mitogenome can be used as an effective mechanism to understand the phylogenetic relationships within Myriapoda.

Keywords: centipedes; *Mecistocephalus marmoratus*; mitogenome; phylogenetic relationship; rearrangement; *Scolopendra subspinipes*

1. Introduction

Centipedes, also known as the Chilopoda (CHI), and their related groups (Diplopoda (DIP), Symphyla (SYM), and Pauropoda (PAU)) comprise the subphylum Myriapoda. Most centipedes are fast-moving, have a predatory lifestyle in terrestrial habitats, and possess poisonous modified maxillipeds [1]. Centipedes comprise more than 3000 species in five extant orders: Scutigeromorpha, Lithobiomorpha, Craterostigmomorpha, Scolopendromorpha, and Geophilomorpha [2,3]. In some studies, *Strigamia maritima* is treated as an ideal model species for ecological and developmental research [4,5]. Recent studies using either comparative morphological or molecular evidence have found that myriapods and all extant myriapod classes are monophyletic [6–20]. Most of these analyses support the contention that the Chilopoda represent the basal lineage of the Myriapoda, with the remaining three classes united as the Progoneata.

However, the phylogenetic relationships among the major extant groups of myriapods remain uncertain, and recent debates have focused on the Edafopoda hypothesis (PAU + SYM group) vs. The Dignatha hypothesis (PAU + DIP group). The Dignatha hypothesis has been universally accepted for more than a century [8,10,20–26], whereas that of Edafopoda is corroborated by nuclear ribosomal genes and some mitochondrial genomes (mitogenomes) [12,14,17]. However, recent phylogenomic analyses based on transcriptomic data showed slightly different results. The results obtained by Szucsich et al,
Benavides et al., and Wang et al. are basically the same, demonstrating strong evidence for the clade Pauropoda + Symphyla (=Edafopoda) as well as for Chilopoda + Diplopoda (=Pectinopoda) [19,21,22]. The difference is that Fernández et al. identified two alternative phylogenetic relationships for Symphyla: one that classifies it as a sister group to the Diplopoda + Chilopoda, and one that places Symphyla closer to Dignatha [27].

The relationships between Chilopoda clades have been elucidated using morphological characteristics and molecular analyses, as follows: (1) the five centipede orders are all monophyletic; (2) the basal division, Scutigeromorpha, is a sister group to the four other centipede lineages; and (3) the Scolopendromorpha + Geophilomorpha comprise the clade Epimorpha [11,27–30]. However, some transcriptome-based phylogenetic analyses did not reveal a sister group relationship between Geophilomorpha and Scolopendromorpha [24], whereas others did [31]. Thus, centipede phylogeny remains a topic that needs further investigation in terms of the position of different centipede lineages within the chilopod orders and the earliest evolutionary splitting events within the centipede lineages.

Arthropod mitogenomes encode 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and a long noncoding region (control region, CR), which have been extensively used to study genetics and evolution at multiple hierarchical levels [31–35]. The order of mitochondrial genes can provide additional phylogenetic information because mitochondrial gene rearrangements are generally rare events. Moreover, most mitochondrial gene arrangements are generally stable in arthropods over a long evolutionary period. Wang et al. found two types of gene orders in the Neuropterida [35]. One was the same as the ancestral mitochondrial gene orders of most insects, and the other was the result of a shuffle of trnC to upstream of trnW (trnC-trnW-trnY), an arrangement that is present in all remaining families of Neuroptera. Song et al. reported that various rearrangements of the hotspot region between the CR and cox1 are found in several insects; all species of the Lepidoptera suborder Ditrysia had the arrangement trnM-trnI-trnQ, and most species of Neuroptera had the transposition of trnW and trnC [34]. Most hymenopterans have the trnI, trnQ, and/or trnM genes in different positions; for example, the trnM-trnI-trnQ order is the most common in Formicidae [36,37].

Although taxon sampling has been limited, the mitogenome has provided evolutionary evidence related to the phylogenetic and evolutionary histories of the Myriapoda. At the same time, a variety of gene rearrangement events have been found. The gene orders in the mitogenomes of Cermatobius longicornis (centipede) and Prionobelum sp. (millipede) are identical to those in Limulus polyphemus (Arthropoda: Xiphosura) [38–41]. All the sequenced mitogenomes of millipedes have a nad6 + cob placement that differs from that of L. polyphemus, except Sphaerotheriida; the pattern of nad6 + cob is believed to be reliable molecular evidence supporting the Helminthomorpha clade, and the inversion of the entire side of a genome (the trnF-nad5-trnH-nad4-nad4L cluster, trnP, the nad1-trnL2-trnL1-rrnL-trnV-rrnS cluster, trnQ, trnC, and trnY) could represent a synapomorphy of a subgroup within Polydesmida [13]. Nine myriapod mitogenomes were compared by Dong et al., who posited that a translocation of trnT from the 5′ end of nad4L was a common event in derived progoneate lineages [12]. Xu et al. sequenced the first Spirobolus mitogenomes and analyzed the phylogenetic relationships within Diplopoda based on 9 orders and 27 species [42].

In previous studies, the mitochondrial gene orders of Scolopendra dehaani, Scolopendra mutilans, S. maritima, Scutigera coleoptrata, and Spirobolus bungii were distinctly different from those of any other myriapod species [42–45]. A high rate of rearrangement makes the Myriapoda an ideal class group for exploring the interactions between gene rearrangements and phylogenetic relationships. Further sequencing of mitogenomes from additional members of the Chilopoda can demonstrate whether such an extensive rearrangement is unique. Common models that attempt to explain gene rearrangement events and investigate the evolutionary implications of these events involve duplication–random loss (TDRL) and duplication–nonrandom loss (TDNL) as the molecular drivers of gene rearrangement [32].
In this study, complete mitogenomes of *M. marmoratus* and *S. subspinipes* were sequenced and annotated, and we used mitogenomes to investigate the gene rearrangement model and the phylogenetic relationships within centipedes, hoping to provide more molecular evidence to explore the relationships within the Myriapoda.

2. Materials and Methods

2.1. Taxon Sampling and Mitochondrial DNA Sequencing

Specimens of *M. marmoratus* and *S. subspinipes* were collected from the Langya mountains, Chuzhou, Anhui province, China (32°16′N, 118°16′E), in July 2014. They were initially preserved in 100% ethanol in the field and were then transferred to −20 °C conditions for long-term storage at the Molecular Biology Laboratory of Chuzhou University (Chuzhou, China). Genomic DNA was extracted from the dehydrated muscle tissues using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). The entire mitogenome was amplified using six primer pairs (Table 1), and all the primers were valid reference primers that had been used for the published species. After using and screening, the six most suitable primer pairs were obtained. Short polymerase chain reaction (PCR) assays (<1.5 kb) were performed using KOD Dash DNA polymerase (Toyobo). The cycling conditions were 94 °C for 5 min; followed by 35 cycles of 30 s at 94 °C, 50 s at 49–52 °C, and 1–2 min at 72 °C (depending on the amplicon size); with a final elongation step at 72 °C for 10 min. Long PCR assays (>1.5 kb) were performed using LA Taq DNA polymerase (TaKaRa). The two-step conditions were as follows: 35 cycles at 96 °C for 2 min and 68 °C for 10 min, followed by incubation at 68 °C for 10 min. The amplified PCR products were electrophoresed on 2% agarose gel, excised, purified, and then analyzed by primer walking on an ABI-PRISM 3730 Automated DNA Sequencer (Applied Biosystems, Waltham, MA, USA).

Table 1. PCR primers used in this study.

| Primer Name | Nucleotide Sequence (5′-3′) | PCR Amplification Product Length | Reference |
|-------------|-----------------------------|---------------------------------|-----------|
| CO1CF       | GCAGCTCTACAAATCATAAGATATTGG  | 0.7 kb                          | [46]      |
| CO1CR       | TAAACTTCAGGGTGACCGAAATCA     |                                 |           |
| Lco1        | TTATAATTTTTTTTTATAGTGATACC   | 3.7 kb                          | [12]      |
| CO3R        | ACATCTACAAAAATGTCAATCCA      |                                 | [47]      |
| Dco3F       | TATCATCTATCAATGAGCAGA        | 3.7 kb                          |           |
| Dn4R        | ATTTATGATTACCTAAGGCTATGG     | 2.9 kb                          | [12]      |
| Hcob        | GCAAATAAAAAATATCTCTGGTTG     |                                 |           |
| DcobF       | ATAATTACGCCTTTCTGGGAT        | 3.4 kb                          |           |
| D12SR       | CTGTGTTCTGATACGATATCCAGTTT   |                                 |           |
| D12SF       | ATATAGGTTATCTAATCTTATCTTCT   | 2.7 kb                          |           |
| Dco1R       | ATGGGGGATATACGGTCATCCCG      |                                 |           |

2.2. Gene Annotation and Secondary Structure Prediction

PCR product sequences were assembled using SeqMan II (DNASTAR Inc., Madison, WI, USA) after checking. Preliminary annotation using the MITOS web server (http://mitos.bioinf.uni-leipzig.de/index.py (accessed on: 10 October 2021)) provided overall information on the mitogenomes [38]. Further annotation of 13 PCGs was performed by identifying their open reading frames and aligning them with homologous genes from other reported myriapod mitogenomes from the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on: 11 October 2021)). tRNA genes were identified by comparing the results predicted using the software programs tRNAscan-SE Search Server v2.0 and ARWEN [48,49]. Based on known gene order information, the boundaries of *rrnL* (16S rRNA) were assumed to be delimited by the ends of the *trnV* and *trnL2* pair. Further, *rrnS* (12S rRNA) was assumed to start from the end of *trnV*, and its end was roughly identified by alignment with the other published millipede sequences. Nucleotide frequencies and codon usage were determined using MEGA X software [50].
2.3. Sequence Alignment and Phylogenetic Analyses

Phylogenetic trees were constructed based on 32 ingroups and 2 outgroups (Table S1), and *Priapulus caudatus* and *Epiperipatus biolleyi* (GenBank accession numbers NC_008557 and NC_009082, respectively) were selected as the outgroups in our analyses. Datasets of 2 rRNA sequences and 13 PCG sequences were selected to analyze the phylogenetic relationships within Myriapoda. The sequences of PCG genes were initially aligned using MASCE v2, and rRNA genes were initially aligned using MAFFT with the E-INS-I strategy [51,52]. Poorly aligned positions were subsequently eliminated using Gblock 9.1b, with default settings [53]. Finally, we used MEGA to concatenate all genes and selected their positions to form two datasets: (1) the PCG12RNA matrix and the first and second codon positions of the 13 PCGs and 2 rRNAs, for a total of 7954 bp; and (2) the AA matrix and the amino acid sequences of 13 PCGs, for a total of 3451 aa.

The optimal partition scheme for each dataset and the best model for each partition were determined using Partition Finder 2 (Table S2), with the Akaike information criterion model and a greedy search algorithm with unlinked branch lengths [54]. We analyzed the phylogenetic relationships using the BI and ML methods using the IQ-TREE and MrBayes v3.2.6 under models, respectively [55,56]. For ML analyses, we used an ultrafast bootstrap approximation approach with $1 \times 10^4$ replicates, whereas for BI analyses, we used the default settings by simulating four independent runs for $1 \times 10^7$–$5 \times 10^7$ generations and sampling every 100 generations after the average standard deviation of split frequencies fell below 0.001. The first 2000 trees were discarded as burn-in. Three replicates of these BI runs were conducted, all of which produced the same topology.

3. Results and Discussion

3.1. Organization of the Mitogenome

As shown in Figure 1 and Tables S3 and S4, the complete mitogenomes of *M. marmoratus* (KX774322) and *S. subspinipes* (MN642577) were sequenced and annotated. The length of the *M. marmoratus* mitogenome was 15,279 bp, and that of *S. subspinipes* was 14,637 bp. Both complete mitogenomes included 37 typical mitochondrial genes—two rRNA genes (*rrnS* (16S rRNA) and *rrnL* (12S rRNA)), 13 PCGs (*cox1-3*, *cob*, *nad1-6*, *nad4L*, *atp6*, and *atp8*), 22 tRNA genes, and a control region (CR) (Tables S3 and S4). The sizes of these two mitogenomes were within the range reported for known myriapod mitogenomes, from 14,487 bp (*Pauropus longiramus*) to 16,833 bp (*C. longicornis*) [13]. The A + T content in the mitogenomes of Chilopoda ranged from 63.4% (*C. longicornis*) to 78.8% (*S. mutilans*). The A + T contents in the mitogenomes of *M. marmoratus* and *S. subspinipes* were 69.5% and 72.7%, respectively. Additionally, both genomes showed an obvious A + T and C + G bias (Table 2).

The length variation was minimal in the PCGs, with greater variation in the putative CR, intergenic overlaps, and tRNAs. Frequent intergenic overlaps (17/37 = 46%) occurred
in the mitogenome of *S. subspinipes*. The two newly sequenced mitogenomes contained the 37 genes commonly found in metazoan mitogenomes as well as a putative CR, including the presumed origin of replication and promoters for transcription initiation. All PCGs started with ATN. The AT contents of the PCGs of Chilopoda ranged from 59.6% (*S. dehaani*) [43] to 77.2% (*S. mutilans*) [41]. The A + T contents of the PCGs in the mitogenomes of *M. marmoratus* and *S. subspinipes* were 67.1% and 71.6%, respectively. Additionally, both genomes showed an obvious T + A bias and a slight C + G bias, except *S. dehaani*, which has an obvious A + T bias, and *S. mutilans*, which has a slight G + C bias (Table 2).

![Circular map of the mitogenomes of *S. subspinipes* and *M. marmoratus*.](image)

**Figure 1.** Circular map of the mitogenomes of *S. subspinipes* and *M. marmoratus*.

### 3.2. Transfer RNAs

The secondary structures of the 22 potential tRNA genes in *M. marmoratus* and *S. subspinipes* were predicted and are shown in Figures S1 and S2, respectively. The newly sequenced mitogenome of *M. marmoratus* revealed the loss of the dihydrouridine arm in *trnC* and *trnS1*. Among the 22 tRNAs in the mitogenome of *S. subspinipes*, *trnT* and *trnP* lacked a TΨC loop, and *trnS1* lacked the DHU arm. Many tRNA genes in the newly sequenced mitogenomes were shortened, with the shortest tRNAs having only 52 nucleotides. A total of 22 tRNAs ranged in length from 52 bp (*trnS1*) to 79 bp (*trnW*) in *S. subspinipes*, and from 52 bp (*trnT*) to 73 bp (*trnN*) in *M. marmoratus*. This difference was mainly caused by the loop region, particularly the variable loop.

### 3.3. Phylogenetic Analyses

As in the previous study, the monophyly of the Myriapoda and the three classes (CHI, DIP, and SYM) were verified using phylogenomic analyses [12,13,19,21,22]. This is considered to be uncontroversial; the current controversy is the phylogenetic relationships among these groups, including PAU. However, the phylogenetic relationships among centipedes reported in previous studies differ due to differences in the molecular markers and analysis methods used [16,27,31,43,44]. Our study provided compelling support for Dignatha (DIP + PAU) being the closest relatives, which supported the relationships among the four groups that were suggested based on morphological and some molecular evidence [24], but was in conflict with the results of phylogenomic analyses with transcriptomic data published by Szucsich et al. and Benavides et al. [19,21]. Regrettably, the position of SYM
in the phylogenetic tree may be unstable due to the limited number of SYM samples used in this study. In our study, we also observed that different results were obtained from different datasets. In the trees produced using the AA matrix (Figure 2a), Chilopoda was identified as the basal lineage of the remaining myriapods and as a sister group with the Progoneata, in both the BI and ML analyses. The relationship within Progoneata was established as (DIP + PAU) + SYM; this result is consistent with earlier results published by Fernández, Edgecombe, and Giribet and is further consistent with the morphological evidence [24]. The results produced using the PCG12RNA matrix were slightly different (Figure 2b). The topology DIP + PAU, with a sister group of SYM + CHI, was identified with high support based on the BI and ML analyses. Thus, in order to clarify the phylogenetic relationships among the major groups of Myriapoda, we need to develop new methods and increase the sampling richness.

Figure 2. Cont.
3.4. Evolution of Gene Rearrangements in the Mitochondrial Genome of Centipedes

Previous studies have shown that the mitogenomes of members of the class Chilopoda are characterized by extensive mitochondrial gene rearrangements [40, 41, 43–45, 58]. In the newly sequenced mitogenomes, the gene arrangement in S. subspinipes was identical to that in L. polyphemus. Within the Scolopendra, S. dehaani and S. subspinipes have the same gene order, except that S. dehaani does not possess trnE and trnL2. Compared with S. dehaani, S. subspinipes has a gene order that is closer to the mitogenomes of other species in the Scolopendromorpha.

Focusing on the Chilopoda, M. marmoratus and S. maritima grouped into one branch, which corresponds to the Geophilomorpha in all trees. In the Scolopendromorpha, the relationships between S. mutilans, S. dehaani, and S. morsitans were closer than the relationship of any of them to S. subspinipes. Contrary to the phylogenetic relationship, the gene orders of the mitogenomes of S. dehaani and S. subspinipes were closer; trnE is missing in S. dehaani. Thus, to verify the relationship between evolution and gene order within the Scolopendromorpha, we need to systematically add more samples to identify the rules of gene rearrangement and clarify the relationships between genera. In previous studies, Scutigeromorpha was identified as a sister group to three other groups, namely Lithobiomorpha,
Geophilomorpha, and Scolopendromorpha [19,27,31]. We found that the relationship between the Lithobiomorpha and Geophilomorpha was closer, although traditional morphological evidence indicates that Geophilomorpha and Scolopendromorpha are more closely related. These relationships identified in the present study are consistent with the conclusions obtained from a previous study [43].

Taxon sampling is essential for the accuracy of phylogenetic inference, and it is important to understand the effect of taxon sampling in a whole-genome or multi-locus phylogenetic study [57]. More Pauropoda should be included, and the selection of different markers will be necessary in future studies to reconstruct a stable phylogenetic topology.

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In order to better understand the evolution of the extensive mitochondrial gene rearrangements of centipedes, the gene orders of published centipede mitogenomes were summarized and mapped on the estimated phylogenetic tree (Figure 3). The gene arrangement of the Lithobiomorpha is relatively conservative, and other classes have been extensively rearranged. At present, the most widely accepted model involves duplication–random loss (TDRL) and duplication–nonrandom loss (TDNL) to explain the molecular drivers of gene rearrangement [59]. A recent study on the pattern of gene rearrangements in Polydesmus sp. GZCS-2019 suggested a new rearrangement model based on three factors: genome-scale duplication, loss, and recombination (TD(N/R) L + C) [21]. This model provides a reference for improving the understanding of the mechanism of gene rearrangement in Myriapoda. However, several unique rearrangement units of M. marmoratus prevent the application of these models to the species.

In this analysis, we assumed that the original mitogenomes of L. polyphemus had an identical gene arrangement, which appears to be ancestral for myriapods and arthropods (Figure 4A). We propose an immature duplication/loss (random and nonrandom) model that resulted in the generation of the mitochondrial gene arrangement of M. marmoratus (Figure 4).

Our hypothesis involves three steps. First, two derivative monomers are arranged in a circular dimer, similar to that found in some myriapods, for example, Narceus annularis, Thyropygus sp., Antrokoreana gracilipes, Trigoniulus coralines, Abacion magnun, Brachycybe lecontii, and Symphyella sp. Subsequently, nonrandom loss occurred according to the transcriptional orientation of each gene. Second, duplication and nonrandom loss events are necessary to explain the translocation of nad3, trnF-nad5-trnH-nad4-nad4L-trnP-trnN, and trnQ-trnM-nad2-trnW (Figure 4B1). In this study, the model of duplication and random loss of genes was used to explain the translocation of the mitochondrial genes trnN, trnL1, and trnl (Figure 4B2) [39]. Thus, the mitochondrial gene arrangement in M. marmoratus was deduced from the original gene order (Figure 4C). Gene density in a duplicated region can be determined via biological constraints rather than by chance. Because mitochondrial gene rearrangements are rare events in animal evolution, they appear to be well-suited for deriving phylogenetic inferences from ancient relationships.
Figure 3. Gene arrangements of the Chilopoda mitochondrial genomes. The mitogenomes have been linearized for ease of comparison and arbitrarily begin with cox1 when possible. Different genes are shown in different colors. Underlined labels indicate that the gene was transcribed from the minority strand.

The highly unusual organization of the mitogenome of S. maritima is possibly due to the stem and loop structures. The rare gene cluster with opposite transcriptional polarity in the mitogenome of M. marmoratus suggests that a nonrandom mechanism is involved in generating this gene order. Based on these two sequenced mitogenomes (S. maritima and M. marmoratus) within Geophilomorpha, gene orders are derived using different mechanisms. In order to explain the reason for the unusual organization of each mitogenome within Geophilomorpha, more mitogenomes need to be sequenced in the future.

The gene transfer and gene block arrangements may represent a synapomorphy in the related lineage, resolving the phylogenetic controversy at multiple hierarchical levels [13]. We believe that meaningful evolutionary information can be obtained by comparing the gene order of myriapod species, provided that data on broader taxon sampling are available. Ultimately, the use of a large number of samples would help elucidate the evolutionary details.
Figure 4. Inferred intermediate steps between *Limulus polyphemus* and *M. marmoratus*. The lost genes are labeled in gray. (A) The ancestral gene arrangement of the myriapod. (B1) Two monomers derived from the duplication of the ancestor arranged in a circular dimer. Subsequently, nonrandom loss occurred according to the orientation of transcription for each gene. (B2) Tandem duplication followed by the random loss of genes and the translocation of tRNAs. (C) Final gene orders of the *M. marmoratus* mitogenome.

4. Conclusions

At present, we report the complete mitogenomes of *M. marmoratus* (15,279 bp) and *S. maritime* (14,637 bp) (Myriapoda: Chilopoda). Both mitogenomes contain 37 typical genes, and the gene order of *S. subspinipes* was the same as that of the original arthropod and myriapod mitogenome (*L. polyphemus*), whereas *M. marmoratus* changed considerably. A simple duplication/loss (random and nonrandom) model was proposed to explain the mitochondrial gene arrangement in *M. marmoratus*; we hope that this model can also reveal mitochondrial gene rearrangement events in other species. Further, we explored the phylogeny of Myriapoda based on mitogenomes. The results suggested the monophyly of the Myriapoda and its main groups and supported Pauropoda and Diplopoda forming the Dignatha, although the relationship between Dignatha and Symphyla needs to be explored further with more systematic evidence. Within the Chilopoda, Scutigeromorpha was a sister group to three other groups: Lithobiomorpha, Geophilomorpha, and Scolopendromorpha. Our study identified a close relationship between Lithobiomorpha and Geophilomorpha. Overall, our results help to better understand the phylogeny of the Myriapoda and explain gene rearrangement events.
Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes13101787/s1: Figure S1: Predicted secondary structure of the 22 tRNAs in the *S. subspinipes* mitogenome (dashes (−) indicate Watson–Crick base pairing); Figure S2: Predicted secondary structure of the 22 tRNAs in the *M. marmoratus* mitogenome (dashes (−) indicate Watson–Crick base pairing); Table S1: GenBank accession numbers for taxa used in this study [12,13,40,41,43–45,47,58–76]; Table S2: Optimal partition strategy and evolutionary models used in phylogenetic analyses; Table S3: Mitochondrial genome organization of *S. subspinipes*; Table S4: Mitochondrial genome organization of *M. marmoratus*.

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References
1. Minelli, A. The Myriapoda, Volume 1, Chapter: Chilopoda. In *The Myriapoda*; Minelli, A., Ed.; Brill: Leiden, The Netherlands, 2011; pp. 1–20.
2. Edgecombe, G.D.; Giribet, G. Evolutionary biology of centipedes (Myriapoda: Chilopoda). *Annu. Rev. Entomol.* 2007, 52, 151–170. [CrossRef] [PubMed]
3. So, W.L.; Nong, W.Y.; Xie, Y.C.; Baril, T.; Ma, H.Y.; Qu, Z.; Haimovitz, J.; Swale, T.; Gaitan-Espitia, J.D.; Lau, K.F.; et al. Myriapod genomes reveal ancestral horizontal gene transfer and hormonal gene loss in millipedes. *Nat. Commun.* 2022, 13, 3010. [CrossRef]
4. Arthur, W.; Chipman, A.D. The centipede *Strigamia maritima*: What it can tell us about the development and evolution of segmentation. *Bioessays* 2005, 27, 653–660. [CrossRef] [PubMed]
5. Brena, C.; Akam, M. The embryonic development of the centipede *Strigamia maritima*. *Dev. Biol.* 2012, 363, 290–307. [CrossRef]
6. Green, J.; Akam, M. Evolution of the pair rule gene network: Insights from a centipede. *Dev. Biol.* 2013, 382, 235–245. [CrossRef] [PubMed]
7. Ax, P. *Ein Lehrbuch der Phylogenetischen Systematik*; Gustav Fischer Verlag: Stuttgart, Germany, 1999; p. 384.
8. Bäcker, H.; Fanenbruck, M.; Wägele, J.W. A forgotten homology supporting the monophyly of Tracheata: The subcoxa of insects and myriapods revisited. *Zool. Anz.* 2008, 247, 185–207. [CrossRef]
9. Bitsch, C.; Bitsch, J. Phylogenetic relationships of basal hexapods among the mandibulate arthropods: A cladistic analysis based on comparative morphological characters. *Zool. Scr.* 2004, 33, 511–550. [CrossRef]
10. Boudreaux, H. Significance of intersegmental tendon system in arthropod phylogeny and a monophyletic classification of Arthropoda. In *Arthropod Phylogeny*; Van Nostrand Reinhold: New York, NY, USA, 1979; pp. 551–586.
11. Chipman, A.D.; Ferrier, D.E.; Brena, C.; Qu, J.; Hughes, D.S.; Schröder, R.; Torres-Oliva, M.; Znassi, N.; Jiang, H.; Almeida, F.C.; et al. The first myriapod genome sequence reveals conservative arthropod gene content and genome organisation in the centipede *Strigamia maritima*. *PLoS Biol.* 2014, 12, e1002005. [CrossRef]
12. Dong, Y.; Sun, H.; Guo, H.; Pan, D.; Qian, C.; Hao, S.; Zhou, K. The complete mitochondrial genome of *Pauropus longigranus* (Myriapoda: Pauropoda): Implications on early diversification of the myriapods revealed from comparative analysis. *Gene* 2012, 505, 57–65. [CrossRef]
13. Dong, Y.; Zhu, L.; Bai, Y.; Ou, Y.; Wang, C. Complete mitochondrial genomes of two flat-backed millipedes by next-generation sequencing (Diplopoda, Polydesmida). *ZooKeys* 2016, 637, 1–20. [CrossRef]
14. Gai, Y.H.; Song, D.X.; Sun, H.Y.; Zhou, K.Y. Myriapod monophyly and relationships among myriapod classes based on nearly complete 28S and 18S rDNA sequences. *Zool. Sci.* 2006, 23, 1101–1108. [CrossRef] [PubMed]
15. Jamieson, B.G.; Jamieson, J.B.G. *The Ultrastructure and Phylogeny of Insect Spermatozoa*; Cambridge University Press: New York, NY, USA, 1987.
16. Regier, J.C.; Shultz, J.W.; Ganley, A.R.; Hussey, A.; Shi, D.; Ball, B.; Zwick, A.; Stajich, J.E.; Cummings, M.P.; Martin, J.W.; et al. Resolving arthropod phylogeny: Exploring phylogenetic signal within 41 kb of protein-coding nuclear gene sequence. *Syst. Biol.* 2008, 57, 920–938. [CrossRef] [PubMed]

17. Regier, J.C.; Shultz, J.W.; Zwick, A.; Hussey, A.; Ball, B.; Wetzer, R.; Martin, J.W.; Cunningham, C.W. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature* 2010, 463, 1079–1083. [CrossRef] [PubMed]

18. Regier, J.C.; Wilson, H.M.; Shultz, J.W. Phylogenetic analysis of Myriapoda using three nuclear protein-coding genes. *Mol. Phylogenet. Evol.* 2005, 34, 147–158. [CrossRef]

19. Szucsich, N.U.; Bartel, D.; Blanke, A.; Böhm, A.; Donath, A.; Fukui, M.; Grove, S.; Liu, S.; Macek, O.; Machida, R.; et al. Four myriapod relatives—But who are sisters? No end to debates on relationships among the four major myriapod subgroups. *BMC Evol. Biol.* 2020, 20, 144. [CrossRef]

20. Zuo, Q.; Zhang, Z.; Shen, Y. Novel mitochondrial gene rearrangements pattern in the millipede *Polydesmus* sp. GZCS-2019 and phylogenetic analysis of the Myriapoda. *Ecol. Evol.* 2022, 12, e8764. [CrossRef]

21. Benavides, L.R.; Edgecombe, G.D.; Giribet, G. Re-evaluating and dating myriapod diversification with phylotranscriptomics under a regime of dense taxon sampling. *Mol. Phylogenet. Evol.* 2022, 178, 107621. [CrossRef]

22. Wang, J.J.; Bai, Y.; Zhao, H.; Mu, R.; Dong, Y. Reinvestigating the phylogeny of Myriapoda with more extensive taxon sampling and novel genetic perspective. *PeerJ* 2021, 9, e12691. [CrossRef]

23. Edgecombe, G.D.; Wilson, G.D.; Colgan, D.J.; Gray, M.R.; Cassis, G. Arthropod cladistics: Combined analysis of histone H3 and U2 snRNA sequences and morphology. *Cladistics* 2000, 16, 155–203. [CrossRef]

24. Fernández, R.; Edgecombe, G.D.; Giribet, G. Phylogenomics illuminates the backbone of the Myriapoda Tree of Life and reconciles morphological and molecular phylogenies. *Sci. Rep.* 2018, 8, 83. [CrossRef]

25. Pocock, R.I. Contributions to our knowledge of the arthropod fauna of the West Indies. Part III. Diplopoda and Malacopoda, with a supplement on the Arachnida of the class Pedipalpi. *Zool. J. Linn. Soc.* 1894, 24, 473–544. [CrossRef]

26. Piegs, O.W. The development and affinities of the Pauropoda, based on a study of *Pauropus silvaticus*. *J. Cell Sci.* 1947, 3, 275–336. [CrossRef]

27. Fernández, R.; Edgecombe, G.D.; Giribet, G. Exploring phylogenetic relationships within Myriapoda and the effects of matrix composition and occupancy on phylogenomic reconstruction. *Syst. Biol. 2016*, 65, 871–889. [CrossRef]

28. Edgecombe, G.D. Morphological data, extant Myriapoda, and the myriapod stem-group. *Contrib. Zool.* 2004, 73, 207–252. [CrossRef]

29. Edgecombe, G.D.; Giribet, G.; Wheeler, W.C. Phylogeny of Henicopidae (Chilopoda: Lithobiomorpha): A combined analysis of morphology and five molecular loci. *Syst. Entomol.* 2002, 27, 31–64. [CrossRef]

30. Giribet, G.; Carranza, S.; Riutort, M.; Baguna, J.; Ribera, C. Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 1999, 354, 215–222. [CrossRef] [PubMed]

31. Benavides, L.R.; Jiang, C.; Giribet, G. Mimopidae is the sister group to all other scolopendromorph centipedes (Chilopoda, Scolopendromorpha): A phylotranscriptomic approach. *Org. Divers. Evol.* 2021, 21, 591–598. [CrossRef]

32. Boone, J.L. Animal mitochondrial genomes. *Nucleic Acids Res.* 1999, 27, 1767–1780. [CrossRef]

33. Cameron, S.L. Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annu. Rev. Entomol.* 2014, 59, 95–117. [CrossRef]

34. Song, F.; Li, H.; Shao, R.; Shi, A.; Bai, X.; Zheng, X.; Heiss, E.; Cai, W. Rearrangement of mitochondrial tRNA genes in flat bugs (Hemiptera: Aradidae). *Sci. Rep.* 2016, 6, 25725. [CrossRef]

35. Wang, Y.; Liu, X.; Garzón-Orduña, I.J.; Winterton, S.L.; Yan, Y.; Aspöck, U.; Aspöck, H.; Yang, D. Mitochondrial phylogenomics illuminates the evolutionary history of Neuropterida. *Cladistics* 2017, 33, 617–636. [CrossRef] [PubMed]

36. Dowton, M.; Cameron, S.L.; Dowavic, J.I.; Austin, A.D.; Whiting, M.F. Characterization of 67 mitochondrial tRNA gene rearrangements in the Hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral. *Mol. Biol. Evol.* 2009, 26, 1607–1617. [CrossRef] [PubMed]

37. Ruiz-Mena, A.; Mora, P.; Montiel, E.E.; Palomeque, T.; Lorite, P. Complete Nucleotide Sequence of the Mitogenome of *Taphina gibber* (Hymenoptera: Formicidae: Dolichoderinae), Gene Organization and Phylogenetic Implications for the Dolichoderinae Subfamily. *Genes* 2022, 13, 1325. [CrossRef]

38. Bernt, M.; Donath, A.; Jühl, F.; Externbrink, F.; Florentz, C.; Fritzsch, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* 2013, 69, 313–319. [CrossRef] [PubMed]

39. Dong, Y.; Xu, J.J.; Hao, S.J.; Sun, H.Y. The complete mitochondrial genome of the giant pill millipede, Sphaerotheriidae sp. (Myriapoda: Diplopoda: Sphaerotheriidae). *Mitochondrial DNA A* 2012, 23, 333–335. [CrossRef] [PubMed]

40. Gai, Y.; Ma, H.; Ma, J.; Li, C.; Yang, Q. The complete mitochondrial genome of *Scolopocryptops* sp. (Chilopoda: Scolopendromorpha: Scolopocryptopidae). *Mitochondrial DNA A* 2014, 25, 192–193. [CrossRef]

41. Lavrov, D.V.; Brown, W.M.; Boone, J.L. A novel type of RNA editing occurs in the mitochondrial tRNAs of the centipede *Lithobius forficatus*. *Proc. Natl. Acad. Sci. USA* 2000, 97, 13738–13742. [CrossRef]

42. Xu, H.; Fang, Y.; Cao, G.; Shen, C.; Liu, H.; Ruan, H. The Complete Mitochondrial Genome of *Scolopinus bungii* (Diplopoda, Scolopobiidae): The First Sequence for the Genus *Scolopinus*. *Genes* 2022, 13, 1587. [CrossRef]
43. Hu, C.; Wang, S.; Huang, B.; Liu, H.; Xu, L.; Hu, Z.; Liu, Y. The complete mitochondrial genome sequence of *Scolopendra mutilans* L. Koch, 1878 (Scolopendromorpha, Scolopendridae), with a comparative analysis of other centipede genomes. *ZooKeys* **2020**, *925*, 73–98. [CrossRef]

44. Sun, L.; Qi, Y.; Tian, X. Analysis of mitochondrial genome of *Scolopendra subspinipes dehaani*. *Tianjin J. Tradit. Chin. Med.* **2018**, *35*, 225–229.

45. Robertson, H.E.; Lapraz, F.; Rhodes, A.C.; Telford, M.J. The complete mitochondrial genome of the geophilomorph centipede *Strigamia maritima*. *PLoS ONE* **2015**, *10*, e0121369. [CrossRef] [PubMed]

46. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mar. Mol. Biol. Biotechnol.* **1994**, *3*, 294–299. [PubMed]

47. Gai, Y.; Song, D.; Sun, H.; Yang, Q.; Zhou, K. The complete mitochondrial genome of *Symphyla* sp. (Myriapoda: Symphyla): Extensive gene order rearrangement and evidence in favor of Progoneata. *Mol. Phylogenet. Evol.* **2008**, *49*, 574–585. [CrossRef] [PubMed]

48. Chan, P.P.; Lin, B.Y.; Mak, A.J.; Lowe, T.M. tRNAscan-SE 2.0: Improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res.* **2021**, *49*, 9077–9096. [CrossRef] [PubMed]

49. Laslett, D.; Canbäck, B. ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics* **2008**, *24*, 172–175. [CrossRef] [PubMed]

50. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]

51. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2019**, *20*, 1160–1166. [CrossRef]

52. Ranwez, V.; Douzery, E.J.; Cambon, C.; Chantret, N.; Delsuc, F. MACSE v2: Toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. *Mol. Biol. Evol.* **2018**, *35*, 2582–2584. [CrossRef]

53. Talavera, G.; Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **2007**, *56*, 564–577. [CrossRef]

54. Lanfear, R.; Frandsen, P.B.; Wright, A.M.; Senfeld, T.; Calcott, B. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **2017**, *34*, 772–773. [CrossRef]

55. Huelsenbeck, J.P.; Ronquist, F. MrBayes: Bayesian inference of phylogenetic trees. *Syst. Biol.* **2007**, *56*, 40–57. [CrossRef]

56. Nabhan, A.R.; Sarkar, I.N. The impact of taxon sampling on phylogenetic inference: A review of two decades of controversy. *Brief. Bioinform.* **2012**, *13*, 122–134. [CrossRef]

57. Negrisolo, E.; Minelli, A.; Valle, G. The mitochondrial genome of the house centipede *Scutigera* and the monophyly versus paraphyly of myriapods. *Mol. Biol. Evol.* **2004**, *21*, 770–780. [CrossRef] [PubMed]

58. Lavrov, D.V.; Boore, J.L.; Brown, W.M. Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: Duplication and nonrandom loss. *Mol. Biol. Evol.* **2002**, *19*, 163–169. [CrossRef]

59. Park, S.J.; Choi, E.H.; Hwang, J.S.; Hwang, U.W. The complete mitochondrial genome of a centipede *Bothropolyx* sp. (Chilopoda, Lithobiomorpha, Lithobiidae). *Mitochondrial DNA Part A* **2016**, *27*, 2268–2269.

60. Gai, Y.; Ma, H.; Sun, X.; Ma, J.; Li, C.; Yang, Q. The complete mitochondrial genome of *Cernatobius longicornis* (Chilopoda: Lithobiomorpha: Henicopidae). *Mitochondrial DNA A* **2013**, *24*, 331–332. [CrossRef]

61. Park, S.J.; Lee, Y.S.; Hwang, U.W. Complete mitochondrial genome of a dogebre millipede *Antrokorana gracilipes* (Diplopoda, Juliformia, Julida), and juliformian phylogeny. *Mol. Cells* **2007**, *23*, 182–191.

62. Brewer, M.S.; Swaﬀord, L.; Spruill, C.L.; Bond, J.E. 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* **2013**, *8*, e68005. [CrossRef]

63. Podsadlowski, L.; Kohlhagen, H.; Koch, M. The complete mitochondrial genome of *Scutigerella causeya* (Myriapoda: Symphyla) and the phylogenetic position of Symphyla. *Mol. Phylogenet. Evol.* **2007**, *45*, 251–260. [CrossRef]

64. Park, S.J.; Lee, Y.S.; Hwang, U.W. The complete mitochondrial genome of the sea spider *Achelia bituberculata* (Pycnogonida, Ammotheidae): Arthropod ground pattern of gene arrangement. *BMC Genom.* **2007**, *8*, 343. [CrossRef] [PubMed]

65. Masta, S.E.; Boore, J.L. Parallel evolution of truncated transfer RNA genes in arachnid mitochondrial genomes. *Mol. Biol. Evol.* **2008**, *25*, 949–959. [CrossRef] [PubMed]

66. Lavrov, D.V.; Boore, J.L.; Brown, W.M. The complete mitochondrial DNA sequence of the horseshoe crab *Limulus polyphemus*. *Mol. Biol. Evol.* **2000**, *17*, 813–824. [CrossRef] [PubMed]

67. Shingate, P.; Ravi, V.; Prasad, A.; Tay, B.H.; Venkatesh, B. Chromosome-level genome assembly of the coastal horseshoe crab (Tachypleus gigas). *Mol. Ecol. Resour.* **2020**, *20*, 1748–1760. [CrossRef]

68. Wilson, K.; Cahill, V.; Ballment, E.; Benzie, J. The complete sequence of the mitochondrial genome of the crustacean *Penaeus monodon*: Are malacostracan crustaceans more closely related to insects than to branchiopods? *Mol. Biol. Evol.* **2000**, *17*, 863–874. [CrossRef]
70. Lavrov, D.V.; Brown, W.M.; Boore, J.L. Phylogenetic position of the Pentastomida and (pan) crustacean relationships. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **2004**, *271*, 537–544. [CrossRef]

71. Podsiadlowski, L. The mitochondrial genome of the bristletail *Petrobius brevistylis* (Archaeognatha: Machilidae). *Insect Mol. Biol.* **2006**, *15*, 253–258. [CrossRef]

72. Cook, C.E.; Yue, Q.; Akam, M. Mitochondrial genomes suggest that hexapods and crustaceans are mutually paraphyletic. *Proc. R. Soc. B Biol. Sci.* **2005**, *272*, 1295–1304. [CrossRef]

73. Clary, D.O.; Wolstenholme, D.R. The ribosomal RNA genes of *Drosophila* mitochondrial DNA. *Nucleic Acids Res.* **1985**, *13*, 4029–4045. [CrossRef]

74. Carapelli, A.; Nardi, F.; Dallai, R.; Boore, J.; Lio, P.; Frati, F. Relationships between hexapods and crustaceans based on four mitochondrial genes. *Crustacean Issues* **2005**, *16*, 295.

75. Webster, B.L.; Copley, R.R.; Jenner, R.A.; Mackenzie-Dodds, J.A.; Bourlat, S.J.; Rota-Stabelli, O.; Littlewood, D.; Telford, M.J. Mitogenomics and phylogenomics reveal priapulid worms as extant models of the ancestral Ecdysozoan. *Evol. Dev.* **2006**, *8*, 502–510. [CrossRef] [PubMed]

76. Ding, J.; Lan, H.; Xu, W.; Chen, Y.N.; Wu, H.; Jiang, H.M.; Wang, J.C.; Wu, Y.B.; Liu, H. Two complete mitochondrial genomes in *Scolopendra* and a comparative analysis of tRNA rearrangements in centipedes. *Mol. Biol. Rep.* **2022**, *49*, 6173–6180. [CrossRef] [PubMed]