INTRODUCTION

Dipteran family Chironomidae have the most abundant species richness among freshwater macroinvertebrates, including more than 6300 species worldwide, even in Antarctica (Kelley et al., 2014; Kim et al., 2016). Since their great species diversity and ability to inhabit different types of water body, chironomid larvae are key bioindicators for freshwater ecosystem monitoring. Several phylogenetic studies have been conducted based on morphological characters or combining genetic markers to reconstruct the evolutionary history of Diamesinae (Diptera: Chironomidae: Diamesinae). However, the evolutionary history of Diamesinae remains uncertain for lack of information. Here, we carried out comparative mitogenomic analysis and phylogenetic analysis of Diamesinae.
of Chironomidae (Brundin, 1966; Cranston et al., 2012; Cranston & Korsch, 2015; Ekrem, 2003; Korsch & Cranston, 2013; Lin et al., 2018; Qi et al., 2019; Sæther, 1977, 2000; Serra-Tosio, 1973; Silva et al., 2015), but few has attempted to use mitogenomes. Diamesinae (Figure 1) is a relatively small subfamily within Chironomidae, containing over 100 species of six tribes: Boreheptagyini, Diamesini, Harrisoniini, Heptagyini, Lobodiamesini, and Protanyiini (Ashe & O’Connor, 2009; Brundin, 1966; Sæther, 2000). At present, Boreheptagyini includes three genera (Boreoheptagyia Brundin, Palatovia Makarchenko & Semenchenco, and Shilovia Makarchenko) distributed in Holarctic and Oriental regions (Makarchenko et al., 2017). Diamesini contains 11 genera: Arctodiamesa Makarchenko, Diamesa Meigen, Kaluginia Makarchenko, Lappodiamesa Serra-Tosio, Pagastia Oliver, Potthastia Kieffer, Pseudodiamesa Goetzhebuer, Pseudokiefferiella Zavrel, Sasayusurika Makarchenko, Sympothastia Pagast, and Syndiamesa Kieffer (Ashe & O’Connor, 2009) distributed in Afrotropical, Holarctic, and Oriental regions. Phylogenetic relationships within Diamesinae are still controversial despite more than 50 years of research. Traditionally, the phylogenetic relationships of Diamesinae were inferred by morphological characters (Brundin, 1966; Sæther, 1977, 2000). Until last decade, the phylogenetic relationship of very limited sets of Diamesinae subgroups has been explored based on a few molecular loci (Cranston et al., 2012; Lencioni et al., 2021; Montagna et al., 2016). However, Boreheptagyini and Protanyiini were missing, and Diamesini taxa were undersampled in their study. Therefore, the phylogenetic relationship within Diamesini and Boreheptagyini was recovered by morphological characters is misleading.

In general, mitogenomes of most insects is a double-strand circular DNA molecule ranging from 14 kb to 20 kb in size, encoding 37 genes (13 protein-coding genes, two ribosomal RNA genes, and 22 transfer RNA genes) and a control region (Boore, 1999; Cameron, 2014; Wolstenholme, 1992). Since its small genome size, maternal inheritance, low sequence recombination, and fast evolutionary rates (Brown et al., 1979; Curole & Kocher, 1999), the mitogenome is considered as powerful marker for phylogenetic and evolutionary analysis in many insect groups (Condamine et al., 2018; Crampton-Platt et al., 2015; Jacobsen et al., 2012; Tang, Zhu, et al., 2019; Yan et al., 2019). Benefiting from the advances of high-throughput sequencing technology, an increasing number of complete mitogenomes have been sequenced among the Diptera (Kang et al., 2016; Li et al., 2020; Miao et al., 2020; Ramakodi et al., 2015; Tang, Yan, et al., 2019; Wang et al., 2021; Yan et al., 2021; Zhang et al., 2022), and have been widely used for mitochondrial structure comparison and phylogenetic analysis at different taxonomic levels (Chen et al., 2018; de Oliveira Aragão et al., 2019; Yan et al., 2019; Zheng et al., 2016; Zhang, Kang, et al., 2019). Prior to this study, rare mitogenomes of Chironomidae were available (Beckenbach, 2012; Deviatiarov et al., 2017; Fang et al., 2022; Jiang et al., 2022; Kim et al., 2016; Kong et al., 2021; Lei et al., 2021; Park et al., 2020; Zhang, Xu, et al., 2019; Zheng et al., 2022; Zheng et al., 2021), limiting our understanding of their mitochondrial structure and phylogenetic pattern. Besides, it is still unknown whether mitogenomes can effectively resolve phylogenetic relationships at different levels within Chironomidae. To date, only one mitogenome of Diamesinae was available, representing Diamesini (Zheng et al., 2021).

In this study, we provide 30 newly sequenced (nearly) complete mitogenomes from 30 species representing Boreheptagyini (four species of one genus) and Diamesini (26 species of five genera) using next-generation sequencing. We analyzed the genomic structure, base composition, substitution, and evolutionary rates among Diamesinae, expanding our knowledge of its diversity of mitogenomes. Coupled with published data, we carried out phylogenetic analysis of Boreheptagyini and Diamesini based on 31 mitogenomes.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling and DNA extraction

Field collection of 30 species were conducted in China during 2014–2020, using classical insect collection techniques such light traps, sweep traps, Malaise traps, and D-nets. Specimens were preserved in ethanol (85% for adults, 95% for immature), and stored at dark at −20°C before morphological and molecular analyses. The total genomic DNA was extracted from thorax of adult and middle larval bodies using a Qiagen DNA Blood and Tissue Kit (Qiagen) following the manufacturer’s protocol. After DNA extraction, the cleared exoskeleton of thorax was mounted in Euparal on microscopy slides together with the corresponding wings, legs, and antennae following the procedures outlined by Sæther (1969). The DNA and vouchers of the species are deposited at the college of Life Sciences, Nankai University, Tianjin, China. Specimens were identified morphologically using relevant taxonomic revisions and species descriptions (Lin, Chang, et al., 2021; Lin, Yu, et al., 2021; Makarchenko et al., 2008, 2021; Makarchenko & Wang, 2017; Moubayed-Breil & Orsini, 2020; Ramakodi et al., 2015; Tang, Yan, et al., 2019; Zhang et al., 2022), and have been widely used for mitochondrial structure comparison and phylogenetic analysis at different taxonomic levels (Chen et al., 2018; de Oliveira Aragão et al., 2019; Yan et al., 2019; Zheng et al., 2016; Zhang, Kang, et al., 2019). Prior to this study, rare mitogenomes of Chironomidae were available (Beckenbach, 2012; Deviatiarov et al., 2017; Fang et al., 2022; Jiang et al., 2022; Kim et al., 2016; Kong et al., 2021; Lei et al., 2021; Park et al., 2020; Zhang, Xu, et al., 2019; Zheng et al., 2022; Zheng et al., 2021), limiting our understanding of their mitochondrial structure and phylogenetic pattern. Besides, it is still unknown whether mitogenomes can effectively resolve phylogenetic relationships at different levels within Chironomidae. To date, only one mitogenome of Diamesinae was available, representing Diamesini (Zheng et al., 2021).

In this study, we provide 30 newly sequenced (nearly) complete mitogenomes from 30 species representing Boreheptagyini (four species of one genus) and Diamesini (26 species of five genera) using next-generation sequencing. We analyzed the genomic structure, base composition, substitution, and evolutionary rates among Diamesinae, expanding our knowledge of its diversity of mitogenomes. Coupled with published data, we carried out phylogenetic analysis of Boreheptagyini and Diamesini based on 31 mitogenomes.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling and DNA extraction

Figure 1  An adult male of Diamesa loeffleri Reiss, 1968 on the ice in Qinghai, China. Photo: Qing-Bo Huo
Thirty mitogenomes were newly sequenced in this study, repre-
senting four species of Boreheptagyiini (four Boreheptagyiia
species) and 26 species of Diamesina (15 Diamesa species, four
Pagastia species, four Potthastia species, two Pseudodiamesa
species and one Sympotthastia species). Since mitogenomes of an-
other four tribes were not available for current molecular study, we
could not reconstruct the phylogeny of the whole subfamily Diamesinae.

Therefore, by integrating one public Potthastia species (GenBank
accession: MW373523), a total of 31 species of Boreheptagyiini and
Diamesina were selected as in-groups. In addition, we selected
one Prodiamesinae species (Prodiamesa olivacea [Meigen, 1818],
GenBank accession: MW373525) and one Orthocladiinae species
(Propsilocerus akamusi [Tokunaga, 1938], GenBank accession:
MW846253) as outgroups for phylogenetic analyses. Detailed in-
formation could be found in Table 1. Each sample ID in Table 1 rep-
resents the voucher unique identifier.

2.2 | Sequencing and mitogenome assembly

The whole genomes were sequenced using the Illumina NovaSeq
6000 platform with 150-bp paired-end reads at Novogene Co.,
Ltd. (Beijing, China). The raw sequencing reads were trimmed with
Trimmomatic (Bolger et al., 2014), and then about two Gb of clean
data were obtained for each sample. The clean data were assembled
using IDBA-UD (Peng et al., 2012) with minimum and maximum k
values of 40 and 120 bp, respectively, and the similarity was set as
98%.

The cytochrome c oxidase I (COI) barcode sequence for each
species was obtained by Sanger sequencing herein and from previ-
ous study (Lin, Yu, et al., 2021), and served as the “bait” references
to acquire the best-fit and targeted mitochondrial contigs by BLAST
(Altschul et al., 1990) search in Geneious 2020.2.1 (Kearse et al.,
2012). Moreover, clean reads were mapped onto the obtained mi-
togenome using Geneious to check the accuracy of the assembly.

2.3 | Genome annotation, composition, and
substitution rate

Genome annotation was conducted following previous study (Zheng
et al., 2020). Transfer RNA (tRNA) genes and their secondary struc-
tures were identified on MITOS2 webserver (available at http://
mitos2.bioinf.uni-leipzig.de/index.py). Ribosomal RNA (rRNA) genes
and protein-coding genes (PCGs) were annotated by aligning with
homologous genes of Potthastia sp. in Geneious. Newly sequenced
mitogenomes were submitted to GenBank (accession numbers:
pending). The mitogenome maps were drawn by the CG View server
V 1.0 (Grant & Stothard, 2008). The base composition, codon usage,
and relative synonymous codon usage (RSCU) values were calcu-
lated in MEGA X (Kumar et al., 2018). The bias of the nucleotide
composition was measured by AT-skew [(A - T)/(A + T)] and GC-
skew [(G - C)/(G + C)]. The ratio (ɷ) of nonsynonymous substitution
rates (Ka) to synonymous substitution rates (Ks) was an excellent
estimator of evolutionary selection pressure. Synonymous substitu-
tion rates (Ks) and nonsynonymous substitution rates (Ka) of mito-
chondrial PCGs were calculated using DnaSP 6.12.03 (Rozas et al.,
2017).

2.4 | Substitution rate and phylogenetic analyses

The level of base substitution saturation for each gene and each po-
sition of the PCGs was assessed using DAMBE 5.6.14 (Xia, 2013).
Substitution of each of the three codon positions are generally not
saturated, except for the transition of 3rd codon positions (Figure
S1). Therefore, the 3rd codon positions of PCGs were excluded for
the phylogenetic analyses. Each gene was aligned using MAFFT
7.402 (Katoh & Standley, 2013) with algorithm G-INS-i strategy. Gap
in each matrix was treated as the fifth character and was retained in
this study. Alignments of individual genes were then concatenated
using SequenceMatrix v1.7.8 (Vaidya et al., 2011), after which three
datasets were prepared for phylogenetic analyses: PCG12 (the 1st
and 2nd codon positions of the 13 PCGs), PCG12R (the 1st and
2nd codon positions of the 13 PCGs and two rRNAs), and third
AA (amino acid sequences of the 13 PCGs). The best partitioning
scheme and best-fit substitution model for each partition was tested
using PartitionFinder 2.0 (Lanfear et al., 2017) with the Bayesian
Information Criterion (BIC). Phylogenetic analyses were conducted
with Maximum likelihood (ML) reconstruction and Bayesian infer-
ence (BI). The ML analysis was performed using IQ-TREE 1.6.10
(Nguyen et al., 2015) with the best-fit substitution model and 1000
bootstrap replicates. BI analysis was performed using MrBayes
3.2.7a (Ronquist et al., 2012) with substitution model in Table S1.
Two simultaneous Markov chain Monte Carlo (MCMC) runs of
10,000,000 generations were conducted, trees were sampled every
1000 generations, and the first 25% of trees discarded as burn-in.
Tracer 1.7 (Rambaut et al., 2018) was used to check convergence
of runs.

3 | RESULTS

3.1 | Mitogenome features of Diamesinae

The mitogenomes of 31 Diamesinae species were included in this
study, 21 of which are complete, with the entire length ranging
from 15,913 bp to 16,411 bp (Table S2). Each mitogenome contains
37 genes (13 PCGs, two rRNAs, and 22 tRNAs) and one control re-
region. Nine PCGs, 14 tRNAs, and 2 rRNAs are coded on the majority
strand (J-strand), while the other genes are coded on the minority
strand (N-strand). The A + T content of the whole mitogenomes
ranged from 72% in Pseudodiamesa sp. 1XL to 77.6% in Potthastia
gaedii (Meigen, 1838) (Figure 2). Among the mitogenomes of
| Sample ID | Subfamily | Species | Life stage | Accession no | Reference |
|-----------|-----------|---------|------------|--------------|-----------|
| XL3436    | Orthocladiinae | Diamesa sp. 1XL | Larva | MZ231027 | This study |
| XL3275    | Diamesinae | Prodiamesa olivacea | Larva | MW372525 | Lin et al. (2022) |
| XL1777    | Diamesinae | Diamesa qiangi | Adult male | MZ12839 | This study |
| ZJ337     | Diamesinae | Diamesa sp. 2XL | Larva | MZ048035 | This study |
| XL3519    | Diamesinae | Diamesa sp. 3XL | Adult male | MZ048036 | This study |
| XL4057    | Diamesinae | Diamesa sp. 4XL | Adult male | MZ048037 | This study |
| CHMIT19   | Diamesinae | Diamesa sp. 5XL | Larva | MZ231028 | This study |
| XL3464    | Diamesinae | Diamesa sp. 6XL | Larva | MZ232924 | This study |
| XL3288    | Diamesinae | Diamesa sp. 7XL | Larva | MZ232929 | This study |
| XL1967    | Diamesinae | Diamesa sp. 8XL | Larva | MZ232930 | This study |
| XL2214    | Diamesinae | Diamesa sp. 9XL | Larva | MZ232931 | This study |
| XL2216    | Diamesinae | Diamesa sp. 10XL | Larva | MZ232932 | This study |

**TABLE 1** Taxonomic information, sampling metadata, GenBank accession numbers, and references of mitochondrial genomes used in the study.
| Sample ID | Species               | Subfamily | Life stage | Accession no | Reference          |
|-----------|-----------------------|-----------|------------|--------------|--------------------|
| XL133     | Diamesinae sp. 1     | Damesiniae | Adult male | MZ043577     | This study         |
| XL1307    | Diamesinae sp. 2     | Damesiniae | Larva      | MZ043578     | This study         |
| XL1312    | Diamesinae           | Damesiniae | Larva      | MZ158295     | This study         |
| XL1321    | Diamesinae           | Damesiniae | Larva      | MZ158292     | This study         |
| XL1336    | Pagastia lanceolata  | Damesiniae | Larva      | MZ000251     | This study         |
| XL1345    | Pagastia sp. 1      | Damesiniae | Larva      | MZ000250     | This study         |
| XL1351    | Pagastia sp. 2      | Damesiniae | Larva      | MZ000250     | This study         |
| XL1367    | Pagastia sp. 3      | Damesiniae | Larva      | MZ000250     | This study         |
| XL1373    | Pagastia sp. 4      | Damesiniae | Larva      | MZ000250     | This study         |
| XL1382    | Pagastia sp. 5      | Damesiniae | Larva      | MZ000250     | This study         |
| ZJ283     | Sympotthastia takatensis | Damesiniae | Pupa      | MZ231026     | This study         |

**TABLE 1 (Continued)**
FIGURE 2 A+T content of mitochondrial genes of Diamesinae species. The X-axis shows the species names and the Y-axis shows the percentage of A+T content.

FIGURE 3 Start and termination codons of PCGs among Diamesinae species. The X-axis shows the names of PCGs and the Y-axis shows the codon numbers.
Diamesinae, the control region and the 3rd codon of PCGs have the highest A + T content, while the 1st and 2nd codons of PCGs exhibit the lowest A + T content. The A + T content of rRNA genes is slightly higher than that in the whole mitogenomes, PCGs, and tRNA genes (Figure 2). In all selected species, the AT-skew value of tRNA genes is positive while that of PCGs is negative. The GC-skew value of rRNA genes and the 1st codon of PCGs are positive, while negative in the whole mitogenomes and the 2nd codon of PCGs (Figure S2). The start codons in most PCGs of the mitogenomes among Diamesinae are ATN (N represents one of four nucleotides, A, T, C, G), while COI and ND1 start with TTG. In addition, ND5 start with GTG in most mitogenomes of Diamesinae (Figure S3). The most prevalent termination codon used in mitogenomes of Diamesinae is TAA, with a small number of PCGs terminate with TAG, TGA, and T- (Figure 3). The total codon numbers, except the termination codons in mitogenomes of Diamesinae range from 3565 to 3735. Leu2, Phe, and Ile are the three most frequently used codon families, each with a number of more than 300. The least frequently used codon family is Cys, with a number less than 50 (Figure S3).

For the entire Diamesinae, the ratio of Ka/Ks (ω) of all the 13 PCGs is less than 0.35, and the ATP8 exhibits the largest Ka/Ks value while the COI has the lowest Ka/Ks value (Figure 4, Table S3). To better understand the evolutionary pattern and the role of selection in Diamesinae species, the values of Ka/Ks were also calculated at congenic level. The Ka/Ks value was quite heterogeneous at congenic level. For individual genes, ATP6 showed a lower Ka/Ks value in Boreoheptagyia, ND1 and ND4L showed a lower Ka/Ks value in Boreoheptagyia and in Pagastia, and the remaining ten showed a lower Ka/Ks value in Diamesa and in Pagastia (Figure 4, Table S3). We also provided the Ka/Ks values of mitochondrial PCGs of Orthocladiinae and Stenochironomus that we previously reported in Table S3, which are higher than that in Diamesinae.

Each mitogenome of Diamesinae contains 22 typical tRNA genes, with A+T content ranging from 74.0% to 77%. The nucleotide skew of tRNA genes among Diamesinae is consistent, exhibiting positive AT-skew and negative GC-skew (Figure 2, Figure S2). Both 12S and 16S rRNA genes transcribe from the minority strand (N-strand).

Among the mitogenomes of Diamesinae, the length of 12S rRNA gene varies from 794 to 815 bp, and the length of 16S rRNA gene varies from 1345 to 1374 bp (Table S2). The A+T content of 125 and 165 rRNA genes ranges from 76% to 78.6% and 80% to 82.8%, respectively. Both 125 and 165 rRNA genes exhibit positive GC-skew in the mitogenomes of Diamesinae (Figure 2, Figure S2). A total of 21 mitogenomes in the present study have the complete control region, varying from 907 to 1309 bp (Table S2). The A+T content of the control region among the mitogenomes of Diamesinae ranges from 87.7% to 93.9% (Figure 2), extremely higher than the whole mitogenomes.

3.2 | Phylogenetic relationships

Generally, six phylogenetic trees constructed by BI and ML analyses are similar in topology, only with the position of Sympotthastia was unstable (Figure 5). The monophyly of the Diamesinae is fully supported across all analyses using different datasets (Figure 5). Within the Diamesinae, two genera-level topologies were inferred from three datasets: (i) (Potthastia + (Boreoheptagyia +Sympotthastia) + (Diamesa + (Pagastia + Pseudodiamesa)))) was inferred from the PCG12 and PCG12R datasets; (ii) (Potthastia + (Boreoheptagyia + (Sympotthastia + (Diamesa + (Pagastia + Pseudodiamesa)))) was inferred from the AA dataset. The topology inferred from the AA dataset had the strongest nodal support. Based on three different datasets, Boreoheptagyini was deeply nested within Diamesini. The monophyly of Boreoheptagyia, Diamesa, Pagastia, Potthastia, and Pseudodiamesa was well supported by mitogenomes.

4 | DISCUSSION

4.1 | Mitogenome features

A total of 31 mitogenomes of Diamesinae are included in this study, of which 10 mitogenomes have incomplete control region by the highly gene duplication (Cameron, 2014; Zhang & Hewitt, 1997). The lengths of 21 complete mitogenomes of Diamesinae range from 15,913 bp to 16,411 bp due to the variation of the control region. The gene number and arrangement of these mitogenomes are conserved, and all genes arranged in the same order as the ancestral insect mitogenome (Clary & Wolstenholme, 1985). The nucleotide composition of the mitogenomes of Diamesinae is biased toward A+T, which is consistent with other published chironomid species (Beckenbach, 2012; Deviatiiarov et al., 2017; Zheng et al., 2021). The mitogenomes of Diamesinae exhibit positive or negative AT-skew and negative GC-skew, the nucleotide bias of these mitogenomes may be related to the asymmetric mutation processes during replication (Hassanin et al., 2005). Most PCGs of mitogenomes of Diamesinae terminated with complete termination codons, except ND4 and NDS in a few mitogenomes, terminated with a single T that may be completed by post-transcriptional polyadenylation (Ojala...
et al., 1981). The ratio of Ka/Ks (ω) is used to assess the evolutionary rate of PCGs in mitogenome (Cheng et al., 2018; Li et al., 2020). The lengths of rRNA genes are inconsistent among Diamesinae species, indicating a relatively high level of variation in these genes. The A+T content of the control region is significantly higher than the whole mitogenome and other regions in mitogenome in Diamesinae, indicating a strong A+T bias in this region.

4.2 Evolutionary rate

We compared the Ka/Ks value between Diamesinae and other subfamilies of Chironomidae. Previous studies reported the Ka/Ks values of mitochondrial PCGs of Orthocladiinae and Stenochironomus (Lin et al., 2022; Zheng et al., 2022), and the Ka/Ks values of each PCG in these chironomids are higher than that in Diamesinae (Table S3), indicating that the mitochondrial genomes of Diamesinae are under stronger purifying selection than other nonbiting midge species (Hurst, 2002). Mitochondrial genome played a central role in animal energy production, and stronger purification selection could enhance their conserved role in energy production (Hassanin et al., 2009; Yuan et al., 2020). The existence of stronger purifying selection in Diamesinae species may exhibit signs of adaptation to life at cold living conditions (high latitude and high altitude) (Makarchenko et al., 2017). Severe habitats generally accumulate more deleterious mutations, and the stronger purifying selection of mitochondrial PCGs in Diamesinae species may help against these deleterious mutations (Sarkar et al., 2020; Wang et al., 2019). In addition, Diamesinae species lives in the cold environment (Lencioni & Rossaro, 2005; Montagna et al., 2016; Sun et al., 2019) and have a small range of activities, which could lead to a lower metabolic rate. Given the correlation between purification selection and metabolic rate has been reported in several species (Chong & Mueller, 2013; Shen et al., 2009; Wang et al., 2019), we hypothesized that the stronger purifying selection in Diamesinae species may also be associated with metabolic requirement.
The evolutionary rate analyses of Diamesinae also provided new insights for the study of species delimitation. The evolutionary rate of COI was generally considered to be consistent with the evolutionary rate of the species itself, so it has been widely used in species delimitation and phylogenetic studies (Havill et al., 2021; Jones et al., 2021). However, for species with lower evolution rate of mitochondrial PCGs, COI barcodes sometimes failed to accurately define the species boundary of Diamesa (Montagna et al., 2016) (E. Stur, pers. comm.). The mitochondrial genome or individual genes with higher evolution rate may be better choices for species delimitation.

4.3 | Phylogenetic analyses

Previous study has revealed that mitogenomes have poor phylogenetic signals at the subfamily level of Chironomidae (Zheng et al., 2021). However, our study reveals that the mitogenomes of Diamesinae are practically useful for phylogenetic inference. In our study, we applied a variety of strategies to explore the phylogenetic relationships of six genera of the Diamesinae using mitogenomic data, and confirmed the monophyly of Diamesinae. According to traditional morphological systematics, Boreheptagyini could be separated from other tribes of Diamesinae by having distinct pubescence, low antennal ratio, and usual dorsocentral and prealar setae in adults (Brundin, 1966; Sæther, 1977; Serra-Tosio, 1973). According the morphological phylogeny, tribe Boreheptagyini is sister to Heptagyini + Lobodiamesini, and Diamesini is sister to Protanypini. However, in the dated molecular phylogeny of the Chironomidae (Cranston et al., 2012), Boreheptagyini was not sampled, and only one Diamesa species was selected. Our result gives a new insight for the systematic status of Boreheptagyini. Serra-Tosio (1973) presented a simplistic analysis of the tribe Diamesini, indicating that the clade Pagastia + Pseudodiamesinae is sister to the clade (Sympathastia + Potthastia) + (Pseudokieffleriella + (Parapothastia + (Onychodiamesa + (Diamesa + Lappodiamesa))). Willassen (2011) presented an unpublished study based on two mitochondrial genetic markers (COX2 and 16S) of all Diamesini genera except Arctodiamesa and Sympothastia in the 18th International Symposium on Chironomidae, and found that the tribe Diamesi is not monophyletic unless Potthastia is removed, and Boreoheptagyia (and Sasayusurika) are sister to the remaining Diamesini. In our study, Potthastia is placed as the oldest of all Diamesinae genera studied here. Our result corresponds very well with Willassen (2011), supporting that Potthastia is not a Diamesini which is contradictory with traditional morphology-based systematics. Moreover, the phylogenetic position of Sympothastia remain unstable based on mitogenomic phylogeny. In general, missing taxa and lacking of informative genetic characters can give a wrong picture of phylogeny estimation (Xi et al., 2016). Therefore, to finally explore the evolutionary history of Diamesinae, a complete resolution will require a comprehensive taxa sampling with the most informative mitochondrial and nuclear markers.

5 | CONCLUSION

In this study, we sequenced 30 mitogenomes representing 30 species of six genera of Boreheptagyini and Diamesini by whole-genome sequencing technologies, and did the first comparative analysis of mitogenome base composition and evolutionary history in Diamesinae. This study showed that mitogenomes of Diamesinae were conserved in structure, gene order, and nucleotide composition. All protein-coding genes in Diamesinae were under stronger purifying selection than those of other nonbiting midge species, which may exhibit signs of adaptation to life at cold living conditions. Mitogenomes could provide new insight into evolutionary history of Diamesinae based on the dated molecular phylogeny.

AUTHOR CONTRIBUTIONS

Xiao-Long Lin: Data curation (lead); Investigation (equal); Methodology (equal); Software (equal); Writing – original draft (lead). Zheng Liu: Funding acquisition (lead); Writing – review & editing (equal). Li-Ping Yan: Investigation (equal); Methodology (equal); Writing – review & editing (equal). Xin Duan: Formal analysis (equal); Software (equal). Wen-Jun Bu: Supervision (equal); Validation (equal). Xin-Hua Wang: Investigation (equal); Supervision (equal); Validation (equal). Chen-Guang Zheng: Formal analysis (equal); Investigation (equal); Writing – original draft (equal); Writing – review & editing (equal).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The new mitogenomes of Boreoheptagyia alulasetosa, Boreoheptagyia brevitaris, Boreoheptagyia kurobrebis, Boreoheptagyia zhengi, Diamesa loeffleri, Diamesa qiangi, Diamesa sp. 1XL, Diamesa sp. 2 XL, Diamesa sp. 3XL, Diamesa sp. 4XL, Diamesa sp. 5XL, Diamesa sp. 6XL, Diamesa sp. 7XL, Diamesa sp. 8XL, Diamesa sp. 9XL, Diamesa sp. 10XL, Diamesa sp. 11XL, Diamesa sp. 12XL, Diamesa tonsa, Pagastia lanceolata, Pagastia sp. 1XL, Pagastia sp. 2XL, Pagastia tianmumontana, Potthastia gaedii, Potthastia sp. 1XL, Potthastia sp. 2XL, Potthastia sp. 4XL, Pseudodiamesa sp. 1XL, Pseudodiamesa sp. 2XL, and Sympothastia...
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