New data on the mitochondrial genome of the winter crane fly (Diptera: Trichoceridae) with phylogenetic analysis

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ABSTRACT
The family Trichoceridae is a small group in Nematocera (Diptera) with 157 known species in the world. In this study, we report a complete mitochondrial genome sequence of a Trichoceridae species, which is a circular molecule of 16,094bp with 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and a 1063bp long non-coding region (control region). Eight gene overlaps ranging from 1 to 8bp in length and 16 small non-coding intergenic spacers ranging from 1 to 77bp in length are found in the mitochondrial genome. The canonical start codons (ATN) for invertebrate mitochondrial genomes are found in 11 PCGs, except for COI which uses TTG and NDS uses GTG. Stop codons of 12 PCGs are invariably complete TAA and TAG, while COII ends with a single thymine stop codon. Twenty-two tRNAs ranges from 64bp to 72bp, and the rRNAs are determined to be 1326bp in length for 16srRNA and 788bp in length for 28srRNA. Phylogenetic analysis reveals that the family Trichoceridae is a sister-group to the remaining Tipulomorpha, and the relationship between the other families is Pediciidae + (Limoniidae + (Cylindrotomidae + Tipulidae)).

Introduction
The family Trichoceridae, commonly called the winter crane fly, is a small group in Nematocera (Diptera) with 157 known species (Krzemińska et al. 2009). The family is divided into six recent genera within two subfamilies: Diazosma Bergroth, 1913, Nothotrachocera Alexander, 1926 and Trichocera Meigen, 1803 referable to the subfamily Trichocerinae; Adura Krzeminska, 2006, Paracladura Brunetti, 1911 and Zedura Krzeminska, 2005 referable to the subfamily Paracladurinae (Krzemińska 1992, 2005, 2006).

Trichocera Meigen is the largest genus in the winter crane fly with more than 110 known species in the world, of which six species are known from China (Alexander 1930, 1933, 1935a, 1935b, 1938; Yang and Yang 1995). It differs from the other genera by the eye having hairs between the ommatidia, the tibial spurs being present, the first tarsi being longer than the second, the vein A2 being short and curved evenly to the margin of the wing, the crossvein m-cu being absent, and the cerci being downward-curved, elongate and sclerotized (Alexander 1981). Two mitochondrial genomes of Trichoceridae have been published (Beckenbach 2012), one of which belongs to the genus Trichocera. In this study, another Trichocera mitochondrial genome is sequenced and analyzed, providing insights into the phylogeny of the infraorder Tipulomorpha.

Materials and methods
The specimen of Trichocera sp. used in this study was collected from Linggongli, Mount Emei, Sichuan, China (29°34′43″N, 103°17′29″E; 1300 m) and stored in the Entomological Museum of Qingdao Agricultural University, China (No. TRI0001). The total genomic DNA was extracted from thoracic muscle of the specimen using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). The fragments of mitochondrial genome were amplified using standard primers for insects (Simon et al. 1994) and were assembled using MEGA7 (Kumar et al. 2016). The complete sequence was annotated by MITOS WebServer (Bernt et al. 2013) with checking for predicted tRNA and protein-coding genes. Phylogenetic tree was reconstructed using the Maximum Likelihood algorithm in MEGA7 software (Kumar et al. 2016).

Results and discussion
The complete mitochondrial genome of Trichocera sp. (GenBank accession no. MW263048) is 16,094bp in length. It contains 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs) and a 1063bp long non-coding region (control region) between the sRNA and tRNAle−tRNAloc−tRNAmet (IQM). The gene order retains the ancestral arrangement for the order Diptera (Clary and Wolstenholme 1985), encoding 23 genes on the majority
strand and 14 genes on the minority strand. Eight gene overlaps are found in the mitochondrial genome, ranging from 1 to 8 bp in length. The longest overlaps exist between tRNA\textsubscript{Trp} and tRNA\textsubscript{Cys}. Sixteen small non-coding intergenic spacers are found in the mitochondrial genome, ranging from 1 to 77 bp in length. The largest non-coding intergenic spacer is between tRNA\textsubscript{Arg} and tRNA\textsubscript{Asn}. The canonical start codons (ATN) for invertebrate mitochondrial genomes (Wolstenholme 1992) are found in 11 PCGs of the mitochondrial genome, while COI and ND5 use uncanonical start codons TTG and GTG respectively. The complete stop codons, TAA and TAG, are found in 12 PCGs of the mitochondrial genome, except for COI using a single thymine stop codon. The entire 22 typical cloverleaf tRNAs in the arthropod mitochondrial genomes are found in the mitochondrial genome, ranging from 64 bp to 72 bp. Most of the tRNAs could be folded into the classic clover-leaf structures, while the DHU arm of the tRNA\textsubscript{Ser(AAG)} gene did not form a stable stem-loop structure.

Table 1. Comparisons of mitochondrial genomes between two Trichocera flies.

| Region                  | Trichocera sp. | Trichocera bimacula |
|-------------------------|----------------|---------------------|
| Number of PCGs          | 13             | 13                  |
| Number of tRNAs         | 22             | 22                  |
| Number of gene overlaps | 8              | 9                   |
| Number intergenic spacers | 16         | 14                  |
| Whole mitogenome        |                |                     |
| A + T%                  | 75.4           | 75.2                |
| G + C%                  | 24.6           | 24.8                |
| AT-skew                 | 0.003          | 0.013               |
| GC-skew                 | −0.154         | −0.177              |
| PCGs(J)                 |                |                     |
| A + T%                  | 71.5           | 71.1                |
| PCGs(N)                 | 75.8           | 74.8                |
| lrRNA                   |                |                     |
| A + T%                  | 80.9           | 81.6                |
| srRNA                   |                |                     |
| A + T%                  | 77.9           | 78.0                |
| tRNA genes              |                |                     |
| A + T%                  | 75.0           | 75.3                |
| Control region          |                |                     |
| A + T%                  | 88.2           | 89.1                |

Note: The AT-bias and GC-bias of PCGs were calculated by the formulae: AT-skew = (A-T)/(A + T), GC-skew = (G-C)/(C + G).

The nucleotide composition of Trichocera sp. mitochondrial genome is biased toward A and T. The overall AT content of the mitochondrial genome is 75.4% (A: 37.8%; T: 37.6%; C: 14.2%; G: 10.4%). For PCGs, the AT content of the N strand genes (75.8%) is higher than that of the J strand genes (71.5%). For rRNAs, the AT content of the lrRNA (80.9%) is slightly higher than that of the srRNA (77.9%). The AT content of the tRNAs is 75.0%. The control region has the highest AT content (88.2%), which is typical of animal mitochondrial genome. The mitochondrial genome has a weakly positive AT-skew and a negative GC-skew on the J-strand. Comparisons of mitochondrial genomes between Trichocera sp. and T. bimacula are listed in Table 1. The proportionate number of nonsynonymous differences (dN) is usually divided by the proportionate number of synonymous differences (dS) to quantify the gene evolutionary rates. The resulting ratio (dN/dS) among Trichocera flies (Figure 1) shows that ATP8 has the fastest evolutionary rate, while COI has the slowest evolutionary rate.

The arrangement of the infraorder Tipulomorpha, containing the families Cylindrotomidae, Limoniidae, Pediciidae, Tipulidae and Trichoceridae, was accepted by Bertone et al. (2008), Dahl (1980), Griffiths (1990), Oosterbroek and Courtney (1995) and Starý (1992). However, the interfamilial relationships in Tipulomorpha are unresolved for a long time. The phylogenetic tree in our study (Figure 2) indicates that the family Trichoceridae is sister-group to the remaining

Figure 1. The resulting ratio (dN/dS) of 13 protein-coding genes (PCGs) of mitochondrial genomes among Trichocera spp.
Tipulomorpha, which was accepted by Bertone et al. (2008), Hennig (1973), Kang et al. (2017), Oosterbroek and Courtney (1995) and Zhang et al. (2016). The relationship between the four families of the superfamily Tipuloidea is as follows: Pediciidae + (Limoniidae + (Cylindrotomidae + Tipulidae)).

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Disclosure statement
No potential conflict of interest was report by the author(s).

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Data availability statement
The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. MW263048. The associated BioProject and BioSample numbers are PRJNA681477 and SAMN16954468, respectively.

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