

## *Tanyptera (Tanyptera) hebeiensis* Yang et Yang (Diptera: Tipulidae) newly recorded from Shandong, China: sequencing and phylogenetic analysis of the mitochondrial genome

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### ABSTRACT

The genus *Tanyptera* Latreille, 1804 is recorded from Shandong Province, China for the first time with *T. (T.) hebeiensis* Yang et Yang, 1988 found in Mount Kunyu, Shandong. In this study, we report the complete mitochondrial genome sequence of *T. (T.) hebeiensis*, representing the first mitochondrial genome of the subfamily Ctenophorinae (Diptera: Tipulidae), which is a circular molecule of 15,888 bp with an AT content of 77.6%. The mitochondrial genome contains 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and a non-coding region. Gene overlaps are found at nine gene junctions, ranging from 1 to 8 bp in length. The canonical mitochondrial start codons for invertebrate mitochondrial genomes are found in 12 PCGs, except for *COI* which uses the uncanonical start codons TCG. Stop codons of 10 PCGs are invariably complete TAA and TAG, while *COII*, *ND4*, and *ND5* end with a single thymine stop codon. Phylogenetic analysis reveals that the Pediciidae is a sister group to the remaining Tipuloidea, the Cylindrotomidae has a sister-group relationship with the Tipulidae, and the Limoniidae is not a monophyletic clade.

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The genus *Tanyptera* Latreille, 1804 is a small group in crane-flies with 27 known species in the world, of which 22 species were known from the Palearctic Region, four species were known from the Oriental Region and only one species was known from the Nearctic Region (Oosterbroek 2020). There were 13 known species of the genus *Tanyptera* from China distributed in the following 13 provinces, municipalities or autonomous regions: Sichuan (3 species), Hubei (3), Fujian (2), Hebei (2), Gansu (2), Shaanxi (2), Zhejiang (1), Jiangxi (1), Heilongjiang (1), Anhui (1), Neimenggu (1), Beijing (1), Ningxia (1). In the present paper, the genus *Tanyptera* is recorded from Shandong Province, China for the first time with the species *T. (T.) hebeiensis* Yang et Yang, 1988 (specimens examined: 10 males 8 males, China, Shandong, Muping, Mount Kunyu, 2019.VII, Chuande Zhao, malaise trap) (Figure 1). The mitochondrial genome of *T. (T.) hebeiensis* is also sequenced and analyzed.

So far a large number of mitochondrial genomes have been widely used for reconstructing phylogenetic relationships in many insect groups (Beckenbach 2012; Timmermans and Vogler 2012; Wang et al. 2012; Caravas and Friedrich 2013; Li et al. 2013; Li et al. 2015; Zhang et al. 2016; Kang et al. 2017; Wang et al. 2017; Zhang et al. 2019). There are about 700 complete or nearly complete lower dipteran

mitochondrial genomes available in GenBank, of which four are from the family Tipulidae (Beckenbach 2012; Zhang et al. 2016; Zhao et al. 2019). However, they are all from the genus *Tipula* Linnaeus, 1758 of the subfamily Tipulinae (Tipulidae). Here, we report the first complete mitochondrial genome sequence of the subfamily Ctenophorinae (Tipulidae), *T. (T.) hebeiensis* mitochondrial genome, which will provide an insight into the phylogeny.

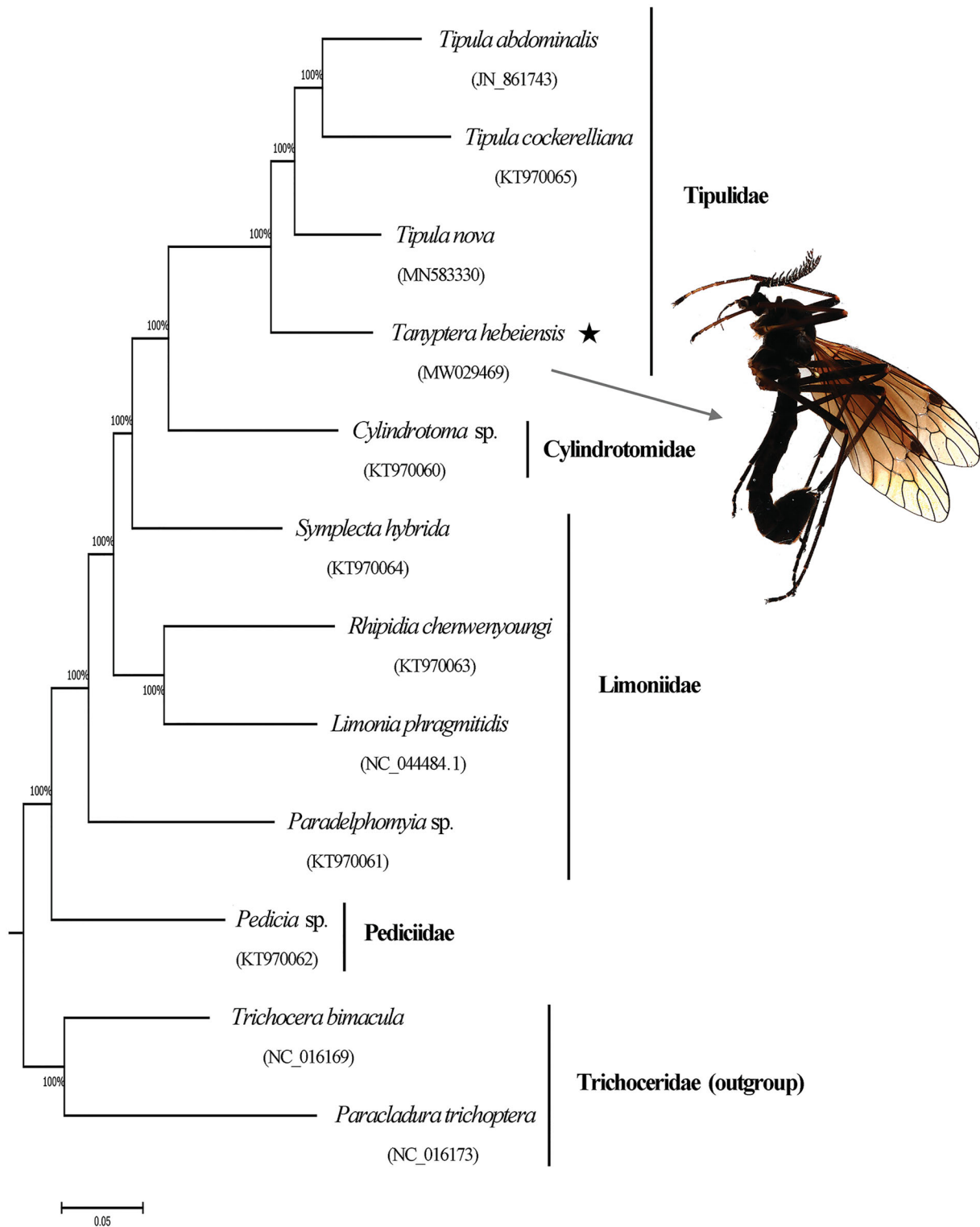
The specimen of *T. (T.) hebeiensis* used in this study was collected from Mount Kunyu in Shandong province, China (37°16'30"N, 121°45'57"E; 200 m) and stored in the Entomological Museum of Qingdao Agricultural University, China (No. TIP0003). The total DNA was extracted from the thoracic muscle using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). The mixed DNA library was constructed with Illumina hiseq by Berrygenomics Co., Ltd (Beijing, China) and the fragment COI was amplified as a 'bait' sequence to identify the target mitochondrial genome (Gillett et al. 2014). The standard PCR reactions were sequenced by primers designed by Simon et al. (2006). BLAST searches were conducted with BioEdit 7.0.5.3 for the bait sequence against mitochondrial genome assemblies. The sequence was annotated following the method proposed by Cameron (2014). The transfer RNA (tRNA) genes were

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**Figure 1.** Phylogenetic tree of Tipuloidea based on whole mitochondrial genomes using maximum-likelihood analysis. Numbers above the branches are bootstrap percentages. GeneBank accession numbers of each species were listed in the tree.

identified with tRNAscan-SE v2.0. The ribosomal RNA (rRNA) genes were detected by alignment with homologous sequences obtained from the published species. The boundaries of protein-coding genes (PCGs) were identified based on open reading frames provided by ORF Finder (<https://www.ncbi.nlm.nih.gov/gorf/gorf.html>). Maximum-likelihood analysis was conducted by MEGA7 (Kumar et al. 2016).

The total length of the mitochondrial genome of *T. (T.) hebeiensis* (GenBank accession no. MW029469) is 15,888 bp and the nucleotide composition of the mitochondrial genome is AT biased (A + T contents: 77.6%). It contains 13 PCGs, 22 tRNAs, 2 rRNAs and a 1065 bp long non-coding region. The gene order shows a conserved arrangement pattern: 23 genes are oriented on the majority strand and 14

genes are transcribed on the minority strand. In the *T. (T.) hebeiensis* mitochondrial genome, gene overlaps are found at nine gene junctions, ranging from 1 to 8 bp in length. The longest overlaps exist between *tRNA<sup>Trp</sup>* and *tRNA<sup>Cys</sup>*. Twelve small non-coding intergenic spacers are found in the *T. (T.) hebeiensis* mitochondrial genome, ranging from 2 to 27 bp in length. The largest non-coding intergenic spacers is between *tRNA<sup>Glu</sup>* and *tRNA<sup>Phe</sup>*. The canonical mitochondrial start codons (ATN) for invertebrate mitochondrial genomes (Wolstenholme 1992) are found in 12 PCGs of the *T. (T.) hebeiensis* mitochondrial genome, except for *COI* which uses the uncanonical start codons TCG. Stop codons of 10 PCGs in *T. (T.) hebeiensis* mitochondrial genome are invariably complete TAA and TAG, while *COII*, *ND4*, and *ND5* end with a single thymine stop codon. Twenty-two typical *tRNAs* in the arthropod mitochondrial genomes are found in the *T. (T.) hebeiensis* mitochondrial genome, ranging from 62 to 72 bp. The length of the *rRNAs* in *T. (T.) hebeiensis* mitochondrial genome are determined to be 1323 bp for *IrrNA* and 791 bp for *srRNA*.

The phylogenetic tree (Figure 1) in this study indicates that the family Pediciidae is sister-group to the remaining Tipuloidea. The sister-group relationship between the families Cylindrotomidae and Tipulidae is strongly supported, which is concordant with Starý (1992), Ribeiro (2008), Petersen et al. (2010), Zhang et al. (2016) and Kang et al. (2017). The family Limoniidae is not supported as a monophyletic clade in this study, which is also accepted by Ribeiro (2008), Petersen et al. (2010) and Zhang et al. (2016).

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

Mitogenome data supporting this study are openly available in GenBank at: <https://www.ncbi.nlm.nih.gov/nucleotide/MW029469>. Associated BioProject, SRA, and BioSample accession numbers are <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA669920>, <https://www.ncbi.nlm.nih.gov/sra/SRR12880699>, and SAMN16481077, respectively. Other GeneBank accession numbers were listed as follows: *Cylindrotoma* sp. (KT970060), *Symplecta (Symplecta) hybrida* (KT970064), *Limonia phragmitidis* (NC\_044484.1), *Paracladura trichoptera*

(NC\_016173), *Paradelphomyia* sp. (KT970061), *Pedicia* sp. (KT970062), *Rhipidia (Rhipidia) chenwenyongii* (KT970063), *Tipula (Nippotipula) abdominalis* (JN\_861743), *Tipula (Acutipula) cockerelliana* (KT970065), *Tipula (Yamatotipula) nova* (MN583330), and *Trichocera bimacula* (NC\_016169).

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