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The mitochondrial genome of one 'twisted-wing parasite' *Xenos* cf. *moutoni* (Insecta, Strepsiptera, Xenidae) from Gaoligong Mountains, Southwest of China

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ABSTRACT

The nearly complete mitochondrial genome (mitogenome) of *Xenos* cf. *moutoni*, one twisted-wing parasite on wasp *Vespa velutina* from Southwest of China, is described in this study. The total length of this mitogenome is 16,717 bp, containing 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs) and an incomplete A + T-rich control region . All of the 13 PCGs are initiated with canonical ATN (N represents A, T, G, C) as start codons; 8 PCGs are terminated with a complete typical stop codon TAA, and the remaining five PCGs (*cox2, cox3, nad3, nad4* and *nad5*) have an incomplete stop codon with just a T. The phylogenetic analysis based on the nucleotide sequences of PCGs and rRNAs indicates that *Xenos* cf. *moutoni* has a close relationship with *Xenos vesparum*, confirming its placement in the family Xenidae.

Strepsiptera, often known as 'twisted wing' parasitoids, is a kind of enigmatic insects with a complex life cycle and unusual morphology (Pohl and Beutel 2008; Cook 2014; Kathirithamby 2018). It is a small order of holometabolous insects, comprising 630 species in 15 families, five of which are extinct (Kathirithamby 2018). All strepsipterans utilize insects as hosts, and their host associations are found in seven insect orders (Cook 2014). Among 10 extant families, the family Mengenillidae use apterygote insect (Zygentoma) as host, and the families, Stylopidae and Xenidae, are known to use aculeate Hymenoptera as hosts: Stylopidae parasitizes bees (Apidae), and Xenidae parasitizes wasps (Crabronidae, Sphecidae, and Vespidae) (Pohl and Beutel 2008; Cook 2014). The Xenidae includes *ca.* 110 described species (*ca.* 19%) and thus is one of the species-rich strepsipteran families (Pohl and Beutel 2008). To date, only three mitogenomic sequences (*Mengenilla australiensis* and *M. moldrzyki* in Mengenillidae and *Xenos vesparum* in Xenidae) were reported (Carapelli et al. 2006; McMahon et al. 2009; Niehuis et al. 2012).

Twisted-wing insect species *Xenos* cf. *moutoni* (males and famales) were collected from the body of wasps (Hymenoptera, Vespidae, *Vespa velutina*) in one nest was collected from Gaoligong Mountains, Xiangda Township, Longling County, Yunnan Province (98°43′26″E, 24°26′38″N), China, on 20 December 2019 by local villagers. With refer-

ence to male morphological traits of strepsipteran insects (Buysson 1903; Kifune and Maeta 1985; Kathirithamby and Engel 2014) and the published strepsipteran mitochondrial *cox1* data (Benda et al. 2019), this species is identified as *Xenos* cf. *moutoni* in the family Xenidae. The voucher specimens (voucher no. KIZ0127514) are stored in Kunming Natural History Museum of Zoology, Chinese Academy of Sciences. Total genomic DNA (gDNA) of one female adult was isolated using a Gentra Puregene Blood kit (Qiagen, Hilden, Germany) based on instructions. Library (150-bp insert size) was prepared and sequenced on Illumina Novaseq 6000 (Novogene, Beijing, China). The 126,444,619 clean reads obtained were used to execute the assembly using both MitoZ v2.3 (Meng et al. 2019) and NOVOPlasty v4.2 (Dierckxsens et al. 2017). Gene annotation was performed using the MITOS2 webserver (Bernt et al. 2013) with manual correction.

Both assembled methods (MitoZ and NOVOPlasty) produced the same sequence except A + T-rich region which is longer in NOVOPlasty assembly than in MitoZ assembly. The mitogenome of *Xenos* cf. *moutoni* (GenBank accession No. MW222190) using NOVOPlasty was 16,717 bp in length and 1,658,750 reads were mapped to the mitogenome with 6,904 mean coverage. The linear mitogenome was considered to be incomplete because its two terminuses lack repetitive

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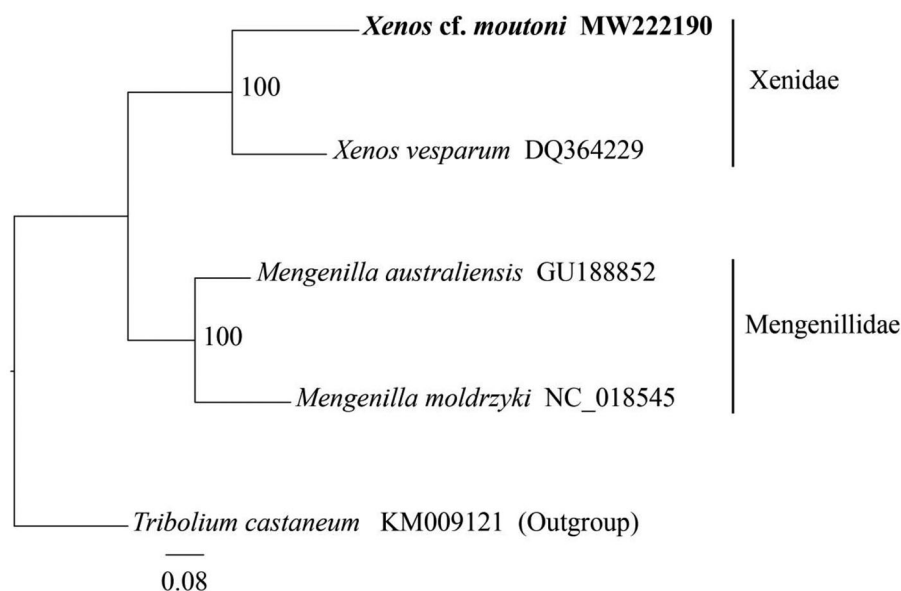*These authors contributed equally to this work.

Figure 1. The phylogenetic tree of four strepsipteran species based on the nucleotide sequence of concatenated 13 protein-coding genes (PCGs) and two ribosomal RNA genes (rRNAs) using maximum likelihood (ML) analysis. Red flour beetle *Tribolium castaneum* was selected as the outgroup. The values at the nodes show the bootstrap support calculated using the maximum likelihood method with 100 replicates.

overlaps with longer than 25 bp (Tang et al. 2014). It contained 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs) and an incomplete control (A + T-rich) region (about 2107 bp). All 13 PCGs are 10,676 bp in length which contain 35.59% A, 9.49% G, 44.30% T and 10.62% C with a strong bias toward A + T (79.89%). All of 13 PCGs use 'ATN' (N represents A, T, G, C) as the start codons; eight of them are terminated with typical stop codon 'TAA', and the other five (*cox2*, *cox3*, *nad3*, *nad4* and *nad5*) have an incomplete stop codon 'T––'. The length of 22 tRNAs ranges from 56 bp to 69 bp and the size of small rRNA (*s-rRNA*) and large rRNA (*l-rRNA*) is 735 bp and 1234 bp, respectively. The arrangement of all 13 PCGs in *Xenos* cf. *moutoni* is consistent with those of two other strepsipterans (*M. australiensis* and *X. vesparum*) and the luminous beetle *R. lufengensis* (Carapelli et al. 2006; Li et al. 2007; McMahon et al. 2009). However, some tRNA gene order changes are observed among the four species in those regions between *nad3* and *nad5*, *nad5* and *nad4* as well as *s-rRNA* and *nad2* and in the location of the *trnL1* (Carapelli et al. 2006; Li et al. 2007; McMahon et al. 2009).

Phylogenetic interference was performed among *Xenos* cf. *moutoni* and other three reported strepsiperan species (Carapelli et al. 2006; McMahon et al. 2009; Niehuis et al. 2012) with *Tribolium castaneum* (Coleopetera, Tenebrionidae) as an outgroup. The 13 PCGs and two rRNAs from these species were aligned and concatenated using MEGA-X (Kumar et al. 2018) and then trimmed the unreliably regions using trimAl v1.4 (gt = 0.5) (Capella-Gutierrez and Silla-Martinez 2009). The phylogenetic tree was constructed based on the concatenated nucleotide sequence using RAxML v8.2.10 (Stamatakis 2014) with the GTR + G + I model. Our result indicates that *Xenos* cf. *moutoni* has a close relationship with *X. vesparum* in the family Xenidae with high bootstrap values (Figure 1), confirming its placement in the family Xenidae.

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. MW222190. The associated BioProject, Bio-Sample and SRA numbers are PRJNA681068, SAMN16898475 and SRR13154212, respectively.

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