**Complete mitochondrial genomes of Anopheles aconitus and Anopheles splendidus and phylogenetics analysis of known mtgenomes in the subgenus Cellia (Diptera: Culicidae: Anophelinae)**

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**ABSTRACT**

The complete mitochondrial genome (mtgenome) sequences of *Anopheles aconitus* and *A. splendidus* were sequenced and analyzed in this study. They are 15,359 bp and 15,362 bp long, respectively and contains 13 protein-coding genes (PCGs), 22 tRNA genes (tRNAs), 2 rRNA genes (rRNAs), and 1 AT-rich control region (CR). The codon UUA (Leu) are predominantly used in all 13 PCGs. ATN is mainly used as the initiation codon in theses PCGs except for COI and NDS genes, which use TCG and GTG as the initiation codon, respectively and TAA as termination codon except for COI, COII, COIII, and ND4 that use the incomplete termination codon T. All of the tRNAs have the typical clover-leaf structure except for tRNA^Ser(AGN), which lost the dihydrouridine (DHU) arm. The CRs have the highest A+T content of 92.97 and 93.18% in these two species, respectively. The phylogenetic relationships of 20 species in the subgenus *Cellia* were constructed using Maximum Likelihood based on concatenated nucleotide sequences of 13 PCGs with the selected best-fit GTR+I+G model. The 20 species are clearly divided into four monophyletic series: Pyretophorus, Neomyzomyia, Neocellia, and Myzomyia, and the Neomyzomyia is basal to the other three series. The Neocellia and Myzomyia are suggested to be sister groups and the Pyretophorus is proposed to sister with Neocellia + Myzomyia. This study provides a basis for further study on mtgenome and phylogenetics in the subgenus *Cellia*.

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et al. (2013) and the PCRs were carried out as described in Hua et al. (2016). The resultant amplified products were verified by electrophoresis on a 1% agarose gel and were sequenced on ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). All fragments were sequenced in both directions.

The complete mtgenomes of An. aconitus and An. splendidus are 15,359 bp (GenBank number KX887320) and 15,362 bp (GenBank number KX887321) in length, respectively. They are both comprised of 37 typical mtgenome genes (13 PCGs, 22 tRNAs, and 2 rRNAs) and a non-coding region. The 22 genes (9 PCGs and 13 tRNAs) are located on the heavy coding strand (H strand), while the other 15 genes (4 PCGs, 9 tRNAs, and 2 rRNAs) are on the light strand (L strand). Seven and nine intergenic spacers exist in the An. aconitus and An. splendidus mtgenomes, which have a total length of 44 bp and 43 bp ranging from 1 bp to 17 bp each, respectively; and the longest intergenic spacer is located between tRNASer(UCN) and ND1. In addition, the 13 PCGs are overlapped with a total length of 29 bp and 30 bp in both An. aconitus and An. splendidus mtgenomes, with the overlaps ranging from 1 bp to 7 bp and the longest two spacers are located between ATP8 and ATP6, and ND4 and ND4L in these two species. The mtgenomes of An. aconitus showed a high nucleotide bias with 78.25% of AT and 21.75% of GC (39.94% A; 38.31% T; 9.19% G; and 12.56% C). The overall nucleotide composition of An. splendidus is 40.18% A, 37.73% T, 9.41% G, and 12.56% C, and CR has the highest AT content (93.18%). The AT contents of rRNAs, tRNAs and PCGs in An. aconitus are 82.02, 78.78, and 76.77% and those of An. splendidus have similar values of 82.02, 78.74, and 76.3%.

The termination codons are the complete termination codon TAA or incomplete termination codon T. Most PCGs have the complete termination codon TAA, whereas four genes (COI, COII, COIII, ND4, and CytB) use ATG as start codon, the start codons of most PCGs follow the typical ATN rules: five genes (COII, ATP6, COIII, ND4, and CytB) use ATG as start codon, three genes (ATP8, ND6, and ND1) use ATT, two genes (ND3 and ND4L) use ATA, and one gene (ND2) use ATC. The use of the GTG start codon has been documented for mtDNA-encoded genes in various organisms, including subgenus Cellia (Krzywinski et al. 2011). The 22 tRNAs of An. aconitus and An. splendidus are 1477 bp and 1489 bp long, respectively, with a range from 64 bp to 72 bp. The 22 tRNAs of An. aconitus and An. splendidus were identified using the program tRNAscan-SE except for tRNASer(AGN) and tRNAArg in comparison with known mosquito mitochondrial sequences. All tRNAs can be folded into typical clover-leaf structure except for tRNA Ser(AGN), which lost the dihydrouridine (DHU) arm. The 20 tRNA genes of An. aconitus and 21 tRNA genes of An. splendidus were identified using the program tRNAscan-SE except for tRNA Ser(AGN) and tRNA Arg in comparison with known mosquito mitochondrial sequences. All tRNAs can be folded into typical clover-leaf structure except for tRNASer(AGN), which lost the dihydrouridine (DHU) arm. The 20 tRNA genes of An. aconitus and 21 tRNA genes of An. splendidus were identified using the program tRNAscan-SE except for tRNA Ser(AGN) and tRNA Arg in comparison with known mosquito mitochondrial sequences. All tRNAs can be folded into typical clover-leaf structure except for tRNA Ser(AGN), which lost the dihydrouridine (DHU) arm. The 20 tRNA genes of An. aconitus and 21 tRNA genes of An. splendidus were identified using the program tRNAscan-SE except for tRNA Ser(AGN) and tRNA Arg in comparison with known mosquito mitochondrial sequences. All tRNAs can be folded into typical clover-leaf structure except for tRNA Ser(AGN), which lost the dihydrouridine (DHU) arm.
respectively and the 16S rRNAs are 1325 bp and 1330 bp long with AT contents 83.4 and 83%, respectively, in the two species. The control region of the An. aconitus and An. splendidus mtgenome are located between 12S rRNA and tRNA\textsuperscript{Ile}. The lengths are 526 bp and 514 bp, with the highest A + T content 92.97 and 93.18%, respectively.

We constructed the phylogenetic relationship of mtgenomes of An. aconitus and An. splendidus and 18 other subgenus Cellia species using Maximum Likelihood (ML) method with Mega 7.0 and with the Anopheles sinensis mtgenome (MF322628) (Ding et al. 2018) as an outgroup. The nucleotide sequences of the 13 PCGs were used in the phylogenetic analysis and the best model GTR + I + G was selected using ModelTest for the analysis. The bootstrap values were calculated based on 1000 replicates and the bootstrap values larger than 50% are noted on the corresponding nodes of the phylogenetic tree (Figure 1). The 18 other subgenus Cellia species of mtgenome sequences were downloaded from GenBank. The result shows that the newly sequenced An. aconitus and An. splendidus are grouped into Myzomyia Series and Neocellia Series, respectively as expected. The 20 species in the subgenus Cellia are clearly divided into four monophyletic Series: Pyretophorus, Neomyzomyia, Neocellia, and Myzomyia with at least 99% bootstrap values of support. The Neomyzomyia Series is basal to other three Series. The Neocellia Series and Myzomyia Series are suggested to be sister groups and the Pyretophorus Series is proposed to sister with Neocellia + Myzomyia clade. These results are consistent with the earlier phylogenetic study result based on morphological and molecular data (Harbach and Kitching 2005; Harbach 2013).

Author contributions
Conceived and designed the research: BC, ZTY. Performed the experiments: ZTY, WBF. Analyzed the data and wrote the paper: ZTY, BC.

Disclosure statement
The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper. It is stated that research meets ethical guidelines.

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