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Sequencing and analysis of the complete mitogenome in *Anopheles culicifacies* species B (Diptera: Culicidae)

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Abstract

The complete mitogenome sequence of *Anopheles culicifacial* species B was sequenced in this study. The length of the mitogenome is 15,330 bp, which contains 13 PCGs, 22 tRNA genes, 2 rRNA genes, and a non-coding control region. The gene order and composition are consistent with those previously reported for other mosquito species. The initiation codon of the PCGs complies with the ATN rule except for *COI* using TCG and *ND5* using GTG as a start codon, and the stop codon is TAA or an only T. The total base composition is 40.4% A, 38.1% T, 12.4% C, and 9.1% G. The phylogenetic tree based on the sequences of 13 PCGs showed that these species were classified into two clades, corresponding to the subgenus *Cellia* and subgenus *Nyssorhynchus. An. culicifacies* species B of Myzomyia Series was clusterd with *An. gambiae* of Pyretophorus Series with a 100% bootstrap value.

Keywords

Mitogenome; sequencing analysis; *Anopheles culicifacies* species B; Culicidae; phylogenetic tree

Anopheles culicifacies Giles is widely distributed in India and neighboring countries, and has been recognized as a complex of five sibling species, A, B, C, D and E. These species are morphologically indistinguishable, whereas exhibit distinct differences in biological characters (Subbarao et al., 1997). All of them except for species B are important vectors of malaria in Southeast Asia (Barik et al., 2009). None of the complete mitogenome of the complex has been reported, and their identification is mainly based on PCR assay that involves in mitochondrial *COII*. In addition to the use as molecular markers for identification, mitogenomes have been widely used in phylogenetics and molecular evolution studies (Logue et al. 2013). In this paper, we report the complete mitogenome of this species, which can provide an important basis for molecular identification and phylogenetic studies of the complex.

The complete mitogenome of *An. culicifacies* species B was successfully sequenced in this study (GenBank number KR732656). This sequencing was based on one female adult, collected from Ja Htu Kong village in the Kachin Region of Myanmar (the border of Yunnan Province in China). The PCR method described in Goswami et al. (2006) was used for the

Declaration of interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper.

species identification. Genomic DNA was extracted from the whole mosquito using the Qiagen DNeasy blood and tissue kit. The complete mitogenome was amplified using 22 primer pairs designed in our Institute, which is in preparation for publication. The control region was cloned with the vector pMD-19T.

The length of the mitogenome of *An. culicifacies* species B is 15,330 bp, which consists of 13 protein-coding, 22 tRNA, 2 rRNA genes and a non-coding control region. Except for the 9 tRNA genes (Gln, Cys, Tyr, Ser (AGN), Phe, His, Pro, Leu (CUN), Val), 4 PCGs (*ND5*, *ND4*, *ND4L*, *ND1*) and 2 rRNA genes, all other genes are encoded on the heavy strand. The gene arrangement is consistent with other mosquito species (Hardy et, al. 2014). The overall nucleotide composition is 40.4% A, 38.1% T, 12.4% C, and 9.1% G. With the exception of *COI* with TCG and *ND5* with GTG as a start codon, all other PCGs are initiated with the standard ATN (ATG, ATT, ATA, and ATC) (Beard et al., 1993). For stop codon, 9 PCGs (*ND2*, *ATP8*, *ATP6*, *ND3*, *ND5*, *ND4L*, *ND6*, *Cytb*, and *ND1*) have the complete TAA, and the other 4 genes (*COI*, *COII*, *COIII*, *ND4*) only have the incomplete T. There are 13 overlap between genes (ranging from 1 to 7 bp) and 9 intergenic spacer regions (ranging from 1 to 17 bp) in the genome. The ribosomal RNAs are relatively conversed as in other insects (Krzywinski et al., 2011). The 16S rRNA is 1,322 bp, located between $tRNA^{Leu}$ and $tRNA^{Val}$ and have an AT content of 83.1%, and the 12S rRNA is 791 bp, flanked by $tRNA^{Val}$ and the control region with an AT content of 80.2%. The 22 tRNAs are totally 1,474 bp in length with gene length ranging from 64 to 72 bp. The total A+T content of 22 tRNAs is 78.6% with $tRNA^{Glu}$ having the highest AT content 92.4% and $tRNA^{Arg}$ the lowest AT content 68.8%. As reported, the anticodens of 22 tRNAs are identical to other anophelines (Krzywinski et al., 2011; Logue et al., 2013). The control region is 498 bp long and has an A+T content of 94%. Compared with other anophelines, this species also contains a conservative T-stretch structure, which is located in position 14,975 bp, totally 18 bp in size (Krzywinski et al., 2011).

We reconstructed the ML phylogenetic tree of the species and 15 other *Anopheles* species using MeGa 5.1, based on the sequences of 13 PCGs with the *Culex pipiens* as outgroup (Figure 1). The 16 *Anopheles* species were classified into two group, corresponding to subgenera *Cellia* and *Nyssorhynchus*. *An. culicifacies* species B of Myzomyia Series was clusterd with *An. gambiae* of Pyretophorus Series with a 100% bootstrap values.

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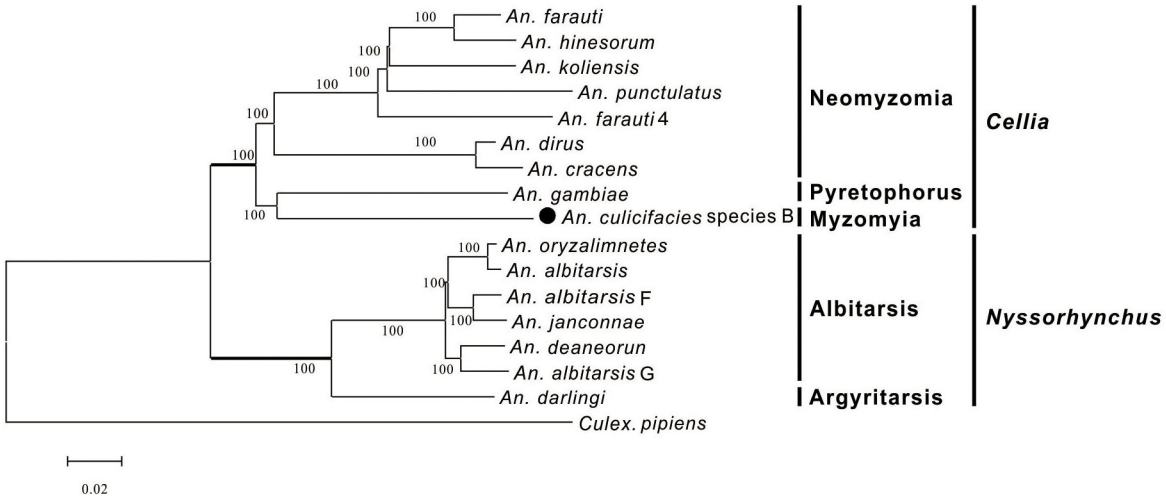

Figure 1.

The maximum likelihood (ML) tree based on PCG's sequences of *An. culicifacies* species B and 15 other *Anopheles* species published. The GTR+G+I model suggested by ModelTest was used, and the bootstrap for 1000 replicates was indicated on each node. Species with GenBank accession number in bracket: *An. farauti* (JX219741), *An. hinesorum* (JX219734), *An. koliensis* (JX219743), *An. punctulatus* (JX219738), *An. farauti* 4 (JX219735), *An. dirus* (JX219731), *An. cracens* (JX219733), *An. gambiae* (NC002084), *An. culicifacies* species B (KR732656), *An. oryzalimnetes* (HQ335345), *An. albitarsis* (HQ335344), *An. albitarsis* F (HQ335349), *An. janconnae* (HQ335348), *An. deaneorun* (NC020663), *An. albitarsis* G (HQ335346), *An. darlingi* (GQ918272), *Culex. pipiens* (NC015079)