

The complete mitochondrial genome of the two-spotted cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) from South Korea

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ABSTRACT

The complete mitochondrial genome of a two-spotted cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) from South Korea is determined and characterized in this study. The circular genome is 16,075 bp long, which consists of 13 protein-coding genes (PCGs), 2 ribosomal RNA genes, 22 transfer RNA genes, and an A + T-rich region. It has a base composition of A (40.35%), G (9.09%), C (16.80%), and T (33.76%). The gene order is identical to the ancestral gene arrangement pattern generally shown in arthropods, with the exception of an inversion of *trnN-trnS1-trnE* into *trnE-trnS1-trnN*. The maximum likelihood (ML) tree supports that *G. bimaculatus* is a distinct member of the monophyletic family Gryllidae.

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Gryllidae; *Gryllus bimaculatus*; mitochondrial genome; molecular phylogeny

The two-spotted cricket *Gryllus bimaculatus* De Geer, 1773, one of the most abundant cricket species, is widely distributed in tropical and subtropical regions of Asia, Africa, and southern Europe (Ragge 1972; Harrison and Bogdanowicz 1995; Mito and Noji 2008). This species belongs to the family Gryllidae within the superfamily Grylloidea (Order Orthoptera), which is known as a general food ingredient in South Korea (i.e. Kim et al. 2020). Generally, *G. bimaculatus* can be easily distinguished from the other species belonging to the genus *Gryllus* by the two spots on the base of its wings. Recently, the complete mitochondrial genome of *G. bimaculatus* was reported from China (Wang et al. 2019). Here, we fully determined the mitochondrial genome of *G. bimaculatus* from South Korea, which was compared with that of Chinese one previously known, with examination of the phylogenetic position within the family Gryllidae.

In this study, a reared specimen of *G. bimaculatus* was obtained from National Institute of Agricultural Sciences, Wanju-gun 55365, South Korea (35°49'45.45"N, 127°02'27.13"E). The specimen is kept in the Kyungpook National University (KNU), Daegu 41566, South Korea (voucher no. KNU2020001) and the extracted genomic DNA is stored in the -80°C in the same KNU depository. The genomic DNA from South Korean specimen was extracted from the whole body using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). The 150 bp paired-end reads were generated by Hiseq X Ten platform (Illumina, San Diego, CA)

sequencing of libraries contacting inserts of ca. 520 bp. The mitochondrial genome was extracted from the whole-genome sequencing data (6.6 Gb read sequences, unpublished data) using the program DeconSeq version 0.4.3 (<http://deconseq.sourceforge.net/>). The raw data of mitochondrial genome were mapped and annotated using the assembler gsMapper version 2.8 (Roche Inc., Basel, Switzerland) with GenBank DB as the reference (e.g. *G. bimaculatus* [MK204367], *Velarifictorus hemelytrus* [NC030762], and *Teleogryllus emma* [NC011823]).

The mitochondrial genome of the South Korean *G. bimaculatus* was completely sequenced, which is 16,075 bp in length (GenBank accession no. MT993975), and contains a standard gene component set including 13 protein-coding genes (PCGs) (*cox1-3*, *cytb*, *nad1-6*, *nad4L*, *atp6*, and *atp8*), 2 ribosomal RNA genes (*rrnL* and *rrnS*), 22 transfer RNA genes, and a non-coding A + T-rich region (CR). Of the 37 typical mitochondrial genes, 21 position on the heavy strand (H-strand) and the remaining 16 on the light strand (L-strand). The overall genome components and gene order are identical to those of the Chinese *G. bimaculatus* published by Wang et al. (2019). In comparison with the ancestral arthropod gene order (*trnN-trnS1-trnE* on the H-strand) (Boore 1999; Woo et al. 2007; Ryu and Hwang 2010; Baek et al. 2014; Park et al. 2016), an inverted gene order (*trnE-trnS1-trnN* on the L-strand) is observed in *G. bimaculatus* from South Korea and China. The inverted feature is known to be

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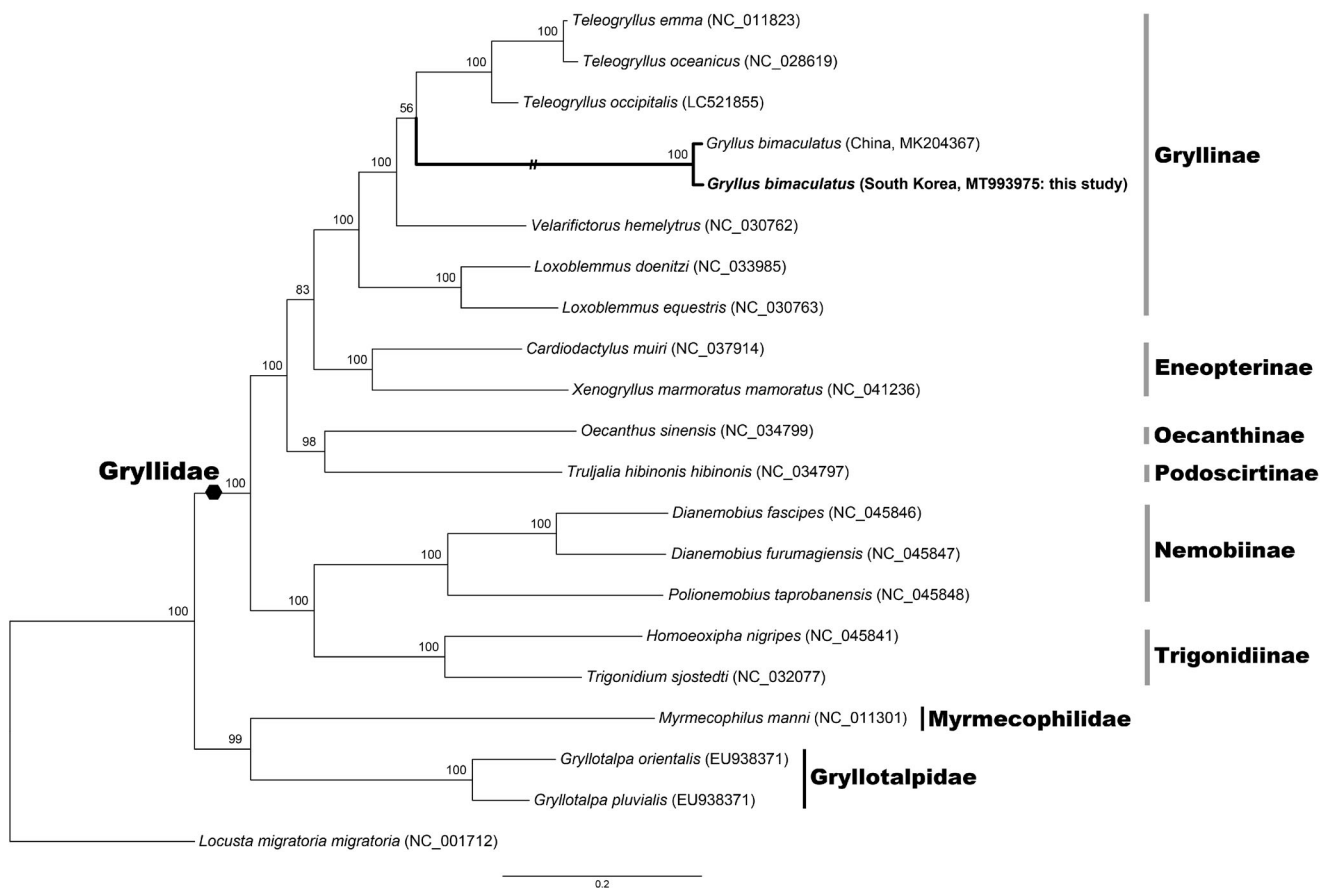


Figure 1. A maximum-likelihood tree reconstructed with the nucleotide sequences of 13 mitochondrial protein-coding genes (PCGs) showing relationships among 20 orthopteran species. It indicates the monophyly of the family Gryllidae, within which two specimens of *G. bimaculatus* from South Korea and China place together. *Locusta migratoria* (Acrididae) is used as an outgroup. Branch supports are inferred from the ultrafast bootstrap method using IQ-TREE web server (Trifinopoulos et al. 2016).

commonly found in all sequenced mitochondrial genomes of the family Gryllidae (Ma and Li 2018). The base composition is 40.35% for A, 9.09% for G, 16.80% for C, 33.7% for T, respectively, which indicates that A+T content (74.11%) is apparently higher than G+C content (25.89%). In all PCGs, the five A+T-biased codons are mainly used: ATT, ATA, AAT, TTA, and AAA. The longest PCG is 1,734 bp for *nad5*, whereas the shortest 156 bp for *atp8*. The common start codon is ATT or ATG, except for *cox1* with TCG and *nad1* with TTG. TAA is the most frequent stop codon, except for the *nad3* and *nad4* with TAG. All tRNAs except *trnS1* lacked a stable dihydrouridine (DHU) arm have the typical clover-leaf secondary structure, which is a common phenomenon, generally found in metazoan mitochondrial tRNAs (Wolstenholme 1992).

To elucidate the phylogenetic position of the two-spotted cricket *G. bimaculatus* within the family Gryllidae, the nucleotide sequences of 13 PCGs were aligned and analyzed from 20 orthopteran mitochondrial genomes, out of which *Locusta migratoria* (Acrididae) was employed as an outgroup. The phylogenetic analysis was conducted by a maximum likelihood (ML) method in IQ-TREE web server (Trifinopoulos et al. 2016) and the best fitting model GTR+I+G4 was selected by ModelFinder (Kalyaanamoorthy et al. 2017). As shown in Figure 1, the South Korean and Chinese specimens of *G. bimaculatus* are grouped together, placing within the monophyletic family Gryllidae. It is expected that this study could be helpful in elucidating genetic diversity between two-spotted crickets

in East Asia including South Korea, China, Japan, etc., and moreover in making a conservation of such edible insect species to become potential food resources for the future.

Disclosure statement

The authors report no conflict of interest.

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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. MT993975. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA704187, SRR13773776, and SANB17487625, respectively.

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