

## The complete mitochondrial genome of the forest crested lizard, *Calotes emma* (Squamata, Agamidae) in China by the next generation sequencing

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### ABSTRACT

The whole mitogenome can prove useful tools for phylogenetic reconstruction and efficiently recover with reasonable taxon sampling. *Calotes emma* is widely distributed and arboreal in habits. However, studies of *C. emma* are still very limited, including population genetics and evolutionary biology. In this study, we reported the complete mitochondrial genome of the *C. emma* by next-generation sequencing for future more researches on systematics and evolution of *C. emma* from the perspective of mitochondrial DNA. The length of mitogenome was 17,688 bp, including 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 tRNA genes and a control region. The phylogenetic tree recovered the monophyly of the *Calotes* and revealed that newly sequenced *C. emma* well supported as the sister taxon to *C. mystaceus* by very high posterior probabilities (1.0). The complete mitochondrial genome of *C. emma* in this study will be helpful for understanding the phylogenetic systematics and relationships, and molecular evolution of *Calotes* in Agamidae.

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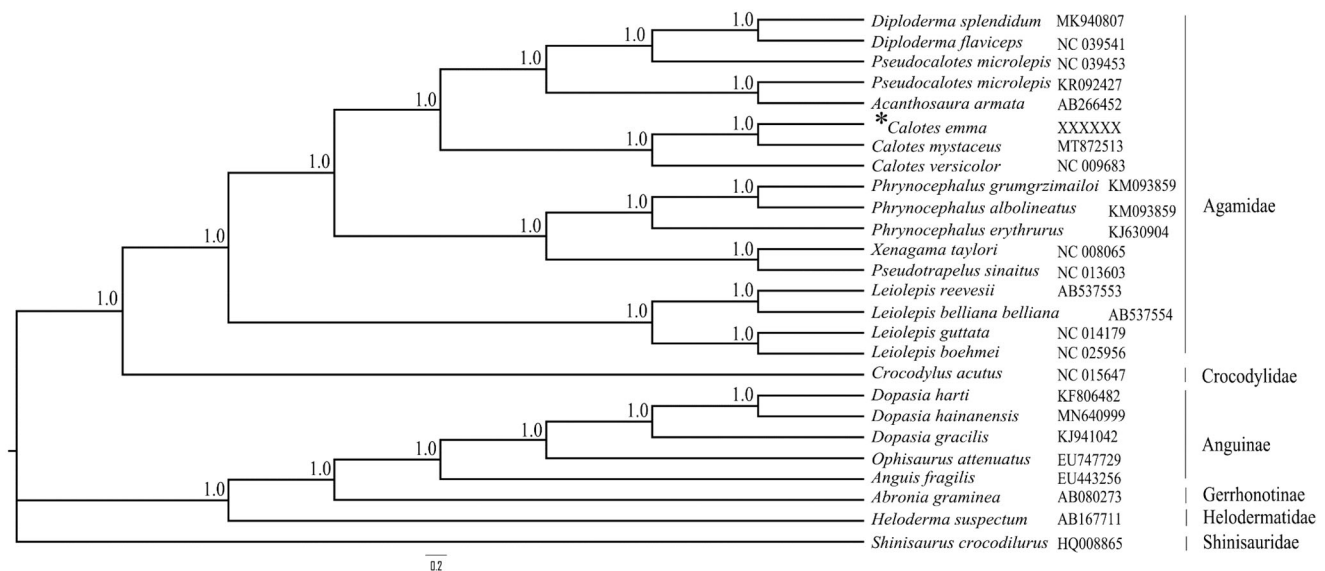
Agamidae; *Calotes emma*; mitochondrial genome; next-generation sequencing; phylogenetic tree

Lizards of the genus *Calotes* Cuvier, 1817, belonging to the family Agamidae in the order Squamata, currently include 26 species distributed from eastern Iran through south China to Sumatra, Indonesia (Vindum et al. 2003; Zug et al. 2006; Krishnan 2008; Hartmann et al. 2013; Amarasinghe, Karunarathna and Fujinuma 2014; Amarasinghe, Karunarathna, Hallermann et al. 2014). The forest crested lizard, *Calotes emma* Gray, 1845, one of the typical members of the genus *Calotes*, is widely distributed across from southern China, India, Vietnam, Burma, Lao PDR, Thailand, Cambodia to Peninsular Malaysia (Zhao et al. 1999; Chan-Ard et al. 2015). This species is diurnal and arboreal in habits, and prefers in tropical forests or open areas under forests at elevations of 80–1950 m (Zhao et al. 1999; Agarwala and Majumder 2015). *C. emma* blend in perfectly with their surroundings, but it is usually found along stream margins of secondary mixed moist deciduous forests and in semi-evergreen forests (Agarwala and Majumder 2015). Grasshoppers, ants, termites, cockroaches, beetles, diverse species of moths and low flying butterflies, and soil-living insects and their larvae are food for *C. emma* (Agarwala and Majumder 2015).

Study of *C. emma* are still very limited, including population genetics and evolutionary biology. To date, there is only one study that high genetic differences among populations of *C. emma* was found throughout Thailand using the mitochondrial CO1 (Saijuntha et al. 2020). Mitochondrial DNA, as an ideal molecular marker, has been widely used in the study of population genetics and evolution. In addition, the whole mitogenome can prove more credible results for

phylogenetic reconstruction and efficiently recover with reasonable taxon sampling than single gene (Rubinstein et al. 2013; Yuan et al. 2016). However, the complete mitogenome sequences of *C. emma* is little known. Thus, in order to provide more data for systematics and evolution of *C. emma* for future researches from the perspective of mitochondrial DNA, in this study, we reported the complete mitogenome of *C. emma* using next-generation sequencing, and then compared with the other agamid lizards which their mitogenomes have been sequenced. It will provide insight into exploring the phylogenetic relationships and evolution of *Calotes* in Agamidae.

The specimen of *C. emma* was sampled from the locality of Pingbian (N22.9617, E103.7917), Yunnan Province, China. Its muscle issue is fixed with 95% ethanol and stored at –20 °C in the herpetological collection, Guangxi University of Chinese Medicine (<http://www.gxtcmu.edu.cn/>, Yong Huang, [huangykiz@163.com](mailto:huangykiz@163.com)) under voucher number 201607241. Total genomic DNA was extracted from the muscle for subsequent analyses using the TIANamp Genomic DNA kit (Tiangen Biotech (Beijing) CO., LTD) and then the complete mitogenome was sequenced by an Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA). We firstly used fastqc (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to evaluate the sequencing results, and then controlled the sequencing quality by ngsqc software. We then performed SPAdes v3.11.0 (Bankevich et al. 2012) to de novo to assemble the 18,425,455 clean reads. Finally, we obtained the complete mitogenome sequence of *C. emma* using the



**Figure 1.** A phylogenetic tree inferred from Bayesian inference of 25 lizards based on 13 protein-coding genes. Node numbers show Bayesian posterior probabilities. The GenBank accession numbers are shown following the names of species.

mitogenome of *C.mystaceus* (GenBank accession number MT872513; Wang et al. 2020) and *C. versicolor* (GenBank accession number NC\_009683; Amer and Kumazawa 2007) as references. The annotation and direction of mitogenome genes were inferred by the MITOS web server (Bernt et al. 2013).

The circular mitogenome of *C.emma* (MZ359215) is 17,118 bp in length, including 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 tRNA genes and a control region. Seven tRNA (tRNA- Gln, Ala, Asn, Cys, Ser, Tyr, and Glu) and Nad6 were encoded on the L-light strand, and the others (12 PCGs and 15 tRNA) were encoded on the H-heavy strand. The overall base composition of the mitogenome is 33.36%A, 27.74%G, 13.52%C and 25.38%T. Eleven PCGs were initiated with the ATG codon, but ND4L begins with ATA and ND5 begins with ATC. Meanwhile, there PCGs (ND1, ND2 and ND6) were ended with TAG codon, five PCGs (COX1, COX2, ATP8, ND4L and Cytb) were terminated with TAA codon, ND4 stopped with AGG, ND5 stopped with AGA, and three PCGs (COX3, ND3 and ATP6) were ended with an incomplete termination base T. The length of D-loop, 12S rRNA and 16S rRNA were 1,444 bp, 829 bp, and 1,524 bp, respectively. The length of 22 tRNA genes ranged from 56 bp in tRNA-Cys to 75 bp in tRNA-Leu.

We constructed phylogenetic analysis using 25 mitogenomes of the Anguinae, Crocodyliidae, Anguinae, Gerrhonotinae and Helodermatidae lizards using *Shinisaurus crocodilurus* (Shinisauridae) as outgroup taxa. We performed the Bayesian inference with GTR + G + I model of nucleotide substitution by MrBayes v.3.2.2 (Ronquist et al. 2012). The most appropriate evolutionary nucleotide substitution model of the 13 PCGs was selected by PartitionFinder 2. The phylogenetic tree (Figure 1) recovered the monophyly of *Calotes* and revealed that newly sequenced *C.emma* well supported as the sister taxon to *C. mystaceus* by very high posterior probabilities (1.0). The complete mitochondrial genome of *C.emma* will be helpful for understanding phylogenetic

systematics and relationships, and molecular evolution of *Calotes* in Agamidae.

## Disclosure statement

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

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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 (<https://www.ncbi.nlm.nih.gov/>) under the accession no. MZ359215. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA773849, SRR16596561, and SAMN22514025 respectively.

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