Next-generation sequencing yields a nearly complete mitochondrial genome of the Forsyth’s toad-headed agama, *Phrynocephalus forsythii* (Reptilia, Squamata, Agamidae)

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**ABSTRACT**

Forsyth’s toad-headed agama, *Phrynocephalus forsythii*, is endemic to the Taklamakan Desert. A nearly complete mitochondrial genome of one individual for this species was determined by next-generation sequencing. The mitogenome is 16,060 bp in length, comprising 2 ribosomal RNA genes, 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and a control region (D-loop). The gene arrangement and composition of *P. forsythii* is identical to the mitogenome of *P. theobaldi* in that *tRNA-Pro* was translocated immediately downstream of *tRNA-Phe*. The D-loop comprised two parts, one (783 bp) existing between *tRNA-Thr* and *tRNA-Phe* and another (with 200 bp already sequenced) inserting between *tRNA-Pro* and 12S rRNA. This mitogenome sequence may provide more data for unveiling the phylogenetic origin and adaptive evolution related to *Phrynocephalus* viviparity.

The traditional protocols to determine the mitochondrial genomes relied on performing either standard/long PCR or cloning, followed by a series of Sanger sequencing (Kumazawa and Endo 2004; Gissi et al. 2008). Recent improvements in next-generation sequencing (NGS) technologies have facilitated the obtainment of mitochondrial genomes (Hahn et al. 2013; Smith 2016).

The Forsyth’s toad-headed agama, *Phrynocephalus forsythii*, is a viviparous species endemic to the Taklamakan Desert, whereas other viviparous congeners species occur on the Tibetan Plateau (Guo and Wang 2007). The phylogenetic placement of this species is still controversial (Jin and Brown 2018), with the *tRNA-Pro* gene being translocated immediately downstream of *tRNA-Phe* gene. The control region consisted of two parts, with part one existing between the *tRNA-Thr* and *tRNA-Phe* (783 bp), while another inserting between the *tRNA-Pro* and 12S rRNA (with 200 bp already sequenced). Most genes are encoded on heavy strand (H-strand) except for *ND6* and eight tRNA genes (*tRNA-Gln, Ala, Asn, Cys, Tyr, Ser[UGA], Glu, and Pro*). Most of the PCGs were terminated with the incomplete termination codon T.

Representative taxa from *Phrynocephalus* with mitogenome sequences available in GenBank plus two Agamidae taxa as outgroup were used to reconstruct the Bayesian phylogenetic tree for evaluating mitochondrial sequence authenticity of *P. forsythii* and its phylogenetic placement. The phylogenetic analysis revealed that monophyly of both genus *Phrynocephalus* and the viviparous group (Figure 1). Three *P. forsythii*
individuals formed a monophyletic group, sister to the remaining viviparous species exclusive of *P. putjatia* and *P. guinanesis*. The mitogenome sequence of *P. forsythii* will provide more data for resolving evolutionary problems related to viviparity and adaptation of *Phrynocephalus*.

**Nucleotide sequence accession number**

The nearly complete mitochondrial genome sequence of *P. forsythii* has been assigned GenBank accession number MK284225.

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No potential conflict of interest was reported by the authors. The authors alone are responsible for the content and writing of this article.

**Figure 1.** A majority-rule consensus tree inferred from Bayesian inference using MrBayes v.3.2.1 (Ronquist and Huelsenbeck 2003) with GTR + r substitution model, based on the PCGs of 22 individuals of toad-headed lizards and two outgroups. DNA sequences were aligned in MEGA v.6.06 (Tamura et al. 2013). The PCGs were translated to amino acids sequences and were manually concatenated all sequences into a single nucleotide dataset (total 11,329 bp). Node numbers show Bayesian posterior probabilities. Branch lengths represent means of the posterior distribution. The novel sequencing sample is highlighted. GenBank accession numbers are given with species/subspecies names.

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