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Complete mitochondrial genome of the endemic legless lizard *Anguis cephallonica* Werner, 1894 and its comparison with mitogenome of *Anguis fragilis* Linnaeus, 1758

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ABSTRACT

Complete mitochondrial genome of the Peloponnese endemic lizard species *Anguis cephallonica* is presented in this study. The complete sequence is 17 208 bp long and consists of 13 protein-coding genes, 22 tRNA genes, two rRNA genes and one control region. The gene order is same as in the relative species *Anguis fragilis*. Length of the 22 tRNA genes varies from 64 bp to 73 bp. The *Anguis cephallonica* mitogenome base composition is: A (30.5%), T (24.2%), C (30.5%), G (14.8%), with an A + T bias (54.7%). Six protein coding genes have incomplete stop codons. This is the first complete mitogenome described in this species as well as in any endemic Peloponnese lizard. Presented complete mitochondrial genome will form a basis for future comparative analysis within the genus *Anguis*.

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Anguis cephallonica; Balkan Peninsula; legless lizards; mitogenome; mtDNA

Legless lizards of the genus *Anguis* Linnaeus, 1758 (slow worms) inhabit a large territory in the Western Palearctic region. Out of five extant species, one is endemic of the Italian Peninsula, two of the Balkans and two other occur in large area of Europe and western Asia (Gvoždík et al. 2010, 2013). The last species, Peloponnese slow worm, *A. cephallonica*, has the smallest range and is endemic to the Peloponnese Peninsula, Zakynthos, Cephalonia and Ithaca islands. Originally, *A. cephallonica* was described as a subspecies of *A. fragilis*, however, precise studies based on morphometric, allozyme and molecular markers resulted into its recognition as a separate species with intraspecific genetic variability (Grillitsch & Cabela 1990; Mayer et al. 1991; Gvoždík et al. 2010, Thanou et al. 2014) and corroborated the Peloponnese Peninsula as an important center of European endemism (cf., Poulakakis et al. 2015).

Complete mitochondrial genomes represent a valuable source of information and can be used to infer phylogenetic relationships of various taxa with more precision and detail than short sequences as has been previously successfully demonstrated (Douglas et al. 2006; Douglas & Gower 2010; Wielstra & Arntzen 2011). In order to provide a comprehensive dataset for future phylogenetic studies we decided to sequence complete mitochondrial DNA (mtDNA) of *A. cephallonica*. We also compared basic genomic characteristics with mitogenome of *A. fragilis*, the only other slow-worm species sequenced so far (Albert et al. 2009).

Tissue sample (muscle from a road-killed individual; voucher number of the Natural History Museum of Crete: NHMC80.3.3.2.) for genetic analysis was collected near Oitylo village (Peloponnese Peninsula, Greece, 36.72086°N; 22.39472°E) (Figure 1). Total genomic DNA was isolated with Sherlock AX (A&A Biotechnology, Gdynia, Poland) according to the product manual, then amplified in three overlapping fragments and sequenced with primer walking method by Wyzer Biosciences Inc. (Cambridge, USA) and assembled with MITOS WebServer (Bernt et al. 2013).

The mitochondrial genome of *A. cephallonica* (17 208 bp; GenBank KU052866) shares identical organization and gene order with *A. fragilis*. Overall base composition of *A. cephallonica* H-strand is as follows: A (30.5%), T (24.2%), C (30.5%), G (14.8%) and slightly differs from *A. fragilis*: A (29.2%), T (26%), C (28.2%), G (16.6%). Twenty-one out of 38 elements vary in length between both genomes. Control region is shorter by 254 nucleotides in *A. cephallonica*. The most of the corresponding start codons are the same for protein coding genes (PCGs) excluding ND3 (ATG – *A. cephallonica*, GTG – *A. fragilis*). Eleven PCGs have the same start codon – ATG, while COX1

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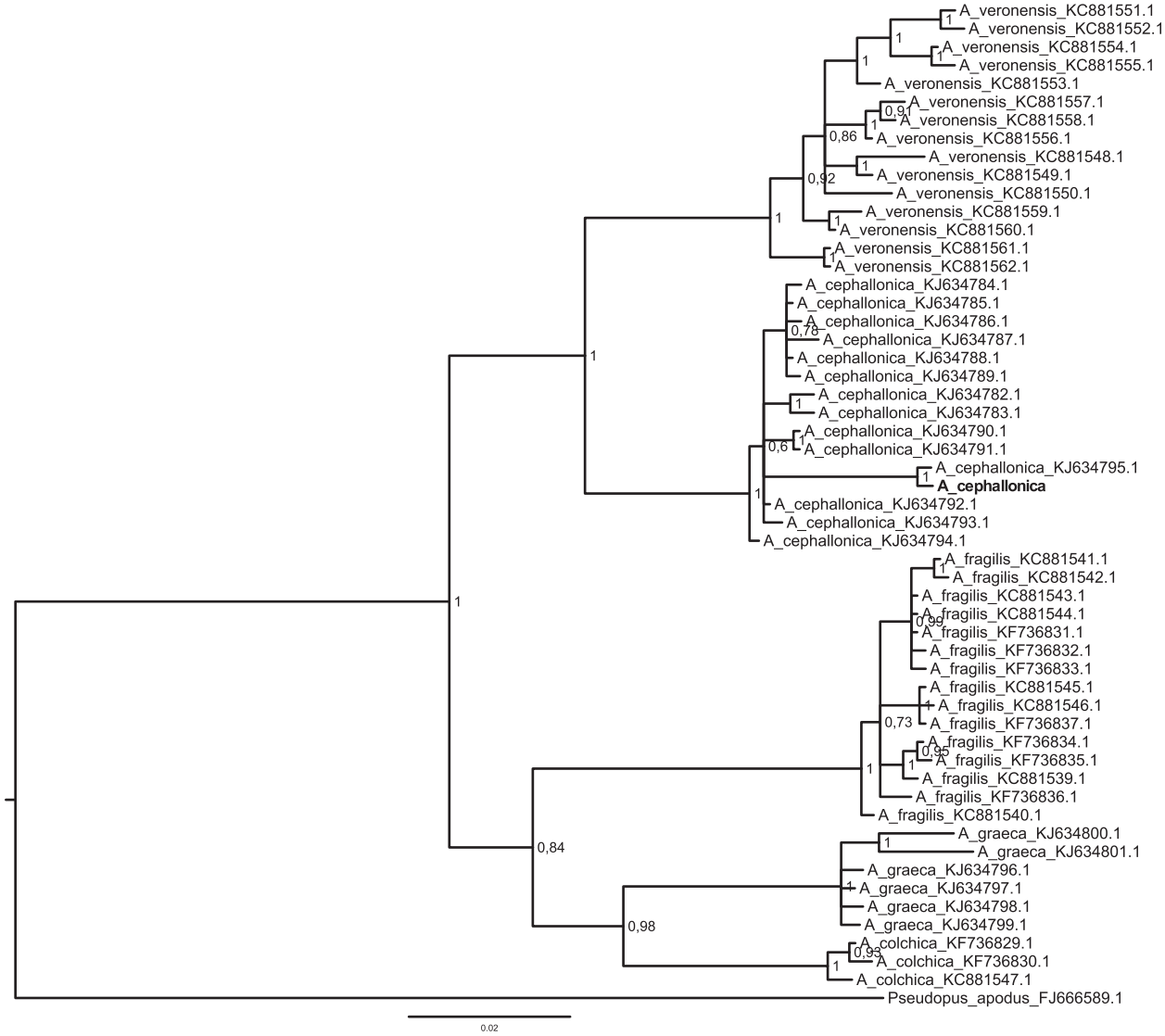

Figure 1. Bayesian phylogenetic tree of five *Anguis* species ND2 sequence (765 bp) alignment.Bayesian phylogenetic tree was generated with. MrBayes 3.2.5 (Ronquist et al. 2012) using model GTR + I + G as suggested by jModelTest 2.1.7 (Guindon & Gascuel 2003; Darriba et al. 2012). 10 000 000 MCMC repetitions with burn-in of 25% was used. ND2 sequence of *Anguis cephallonica* from the present study is bolded and grouped together with other *A. cephallonica* representatives. Tree was rooted with *Pseudopus apodus* ND2 sequence and all GenBank accession numbers of all used sequences are present on the tree. Bayesian posterior probabilities are showed with nodes.

and *A. fragilis* ND3 require GTG. In *A. cephallonica* genome, six of PCGs use truncated stop codons (ATP6, COX2, COX3, ND3, ND4, ND5), three genes stop with TAA (ATP8, ND1, ND4L), three with TAG (COX1, CYTB, ND2) and one with AGA (ND6). When compared to *A. fragilis* genome, stop codons usage differs as the most common stop codons in *A. fragilis* is TAA (ATP6, ATP8, COX1, ND2, ND4L, ND5), then truncated stop codons (COX2, COX3, ND3, ND4) and TAG (CYTB, ND1) and AGA (ND6).

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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