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Complete mitochondrial genome of the Greek nine-spined stickleback *Pungitius hellenicus* (Gasterosteiformes, Gasterosteidae)

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ABSTRACT

The complete mitochondrial genome of the Greek nine-spined stickleback *Pungitius hellenicus* was obtained using Illumina high-throughput sequencing of genomic DNA. The genome was 16 713 bp long, and contained 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and a control region. The arrangement of the genes was identical to that of other Gasterosteidae fishes. However, the control region of *P. hellenicus* contained three copies of imperfect repeated sequences (72–78 bp in single motifs), while *P. pungitius* and *P. tymensis* have one or two copies. Nucleotide identity between *P. hellenicus* and three other *Pungitius* species across all the 37 genic regions was 93.0% to 95.5%.

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Gasterosteidae; genome; mtDNA; *Pungitius hellenicus*

The number of valid species in the circumpolarly distributed teleost fish genus *Pungitius* remains uncertain, but morphological evidence suggests that the Greek nine-spined stickleback (*Pungitius hellenicus*) is likely to be a valid species (Keivany et al. 1997; Keivany & Nelson 2000). However, little genetic information is available on this species (Geiger et al. 2014). Hence, access to the complete mitochondrial genome sequence of *P. hellenicus* would allow evaluation of its validity as a distinct species, as well as its phylogenetic positioning within the genus *Pungitius* (cf., Takahashi & Goto 2001; Keivany & Nelson 2004; Mattern 2004). As the known distribution of *P. hellenicus* is confined to only a few localities in the Sperchios River basin in Central Greece (Keivany et al. 1999; Keivany & Nelson 2000), and the species is listed as critically endangered (Martins & Wiswedel 2015), the genomic information is likely to support conservation and management planning of this species.

Pungitius hellenicus was collected from the Sperchios River basin in Greece (38°51'N, 22°26'E), and genomic DNA was sequenced using the Illumina HiSeq2000 platform with 100 paired-end strategy. In total, 16.6 million reads were aligned against the reference *P. sinensis* mitogenome (Hwang et al. 2012a) using bwa-0.5.10 (Li & Durbin 2009). Mean sequence coverage across the mitogenome was 43-fold, and with the exception of a 265 bp gap in the control region, all regions had at least one-fold coverage (83.7% with ≥20-fold coverage). Sanger sequencing was used to fill the 265 bp gap. PCR products were obtained using a primer set (PCR-F: 5'-CAAGGT TGAACATCTTTCGC-3' and PCR-R: 5'-CTGATACCAGCTCCTGT

CC-3') following the protocols and procedures described in Teacher et al. (2011). The consensus sequence of the *P. hellenicus* mitogenome was generated using SAMtools 1.2 (Li et al. 2009) and manually checked.

The complete mitochondrial genome of *P. hellenicus* was 16 713 bp long (GenBank Accession No. KU236383), containing 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and a control region. The arrangement of the genes was identical to that of other Gasterosteidae fishes (Miya et al. 2001; Kawahara et al. 2009; Hwang et al. 2012a, 2012b). In the 13 protein-coding genes, an incomplete stop codon was found for four genes (*ND2*, *COII*, *ND4* and *Cytb*). Three copies of imperfect repeated sequences (72–78 bp in single motifs) were identified in the control region, whereas one or two copies have been observed in *P. pungitius* and *P. tymensis* (Takahashi & Goto 2001). The overall base composition of the entire mitogenome was 27.4% for A, 26.4% for T, 17.6% for G and 28.6% for C.

To investigate the phylogenetic position of *P. hellenicus* in Gasterosteidae fishes, a maximum-likelihood tree was constructed with the 37 genes (15 581 bp in total) using RAXML v.8.0 under the GTR+GAMMA model, 37 gene partitions and 100 thorough bootstrap replicates (Stamatakis 2014). *Pungitius hellenicus* was phylogenetically positioned with other *Pungitius* species (i.e. *P. pungitius*, *P. sinensis* and *P. kaibarae*), showing clear divergence from them (Figure 1). Nucleotide identity in the 37 gene regions was 93.0% to 95.5% between *P. hellenicus* and the other *Pungitius* species.

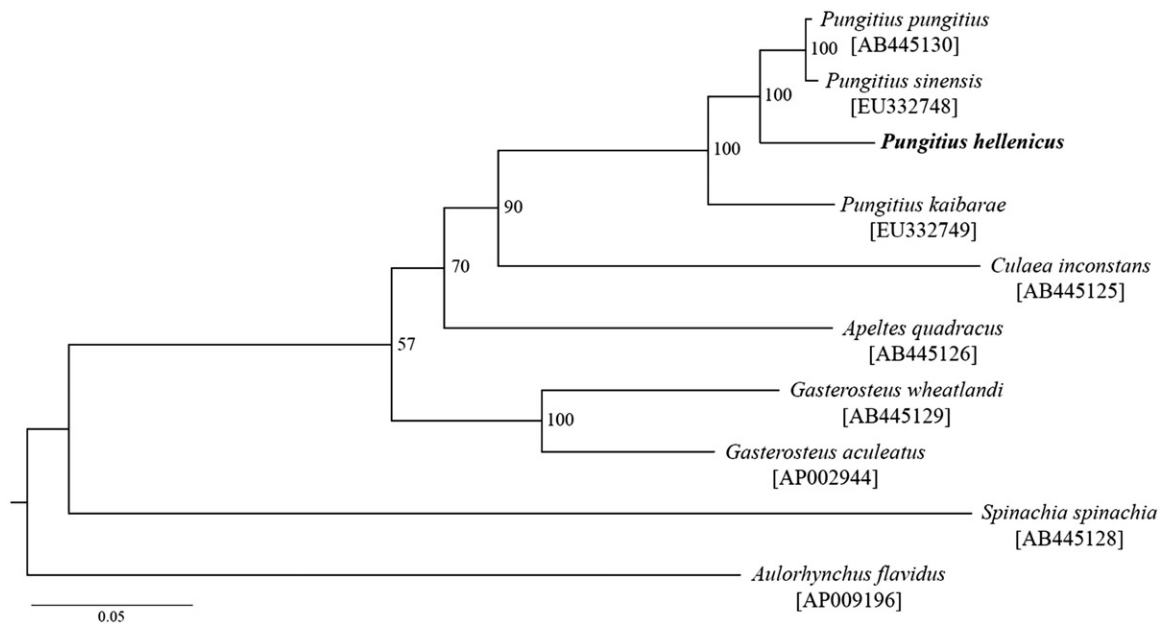


Figure 1. A maximum likelihood tree inferred from 37 mitochondrial genes among nine Gasterosteidae and an outgroup (*Aulorhynchus flavidus*) species. Bootstrap support is indicated at nodes. GenBank accession numbers are indicated in brackets.

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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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