

MITOGENOME ANNOUNCEMENTS

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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%.

ARTICLE HISTORY

Received 8 December 2015
Accepted 22 December 2015

KEYWORDS

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.

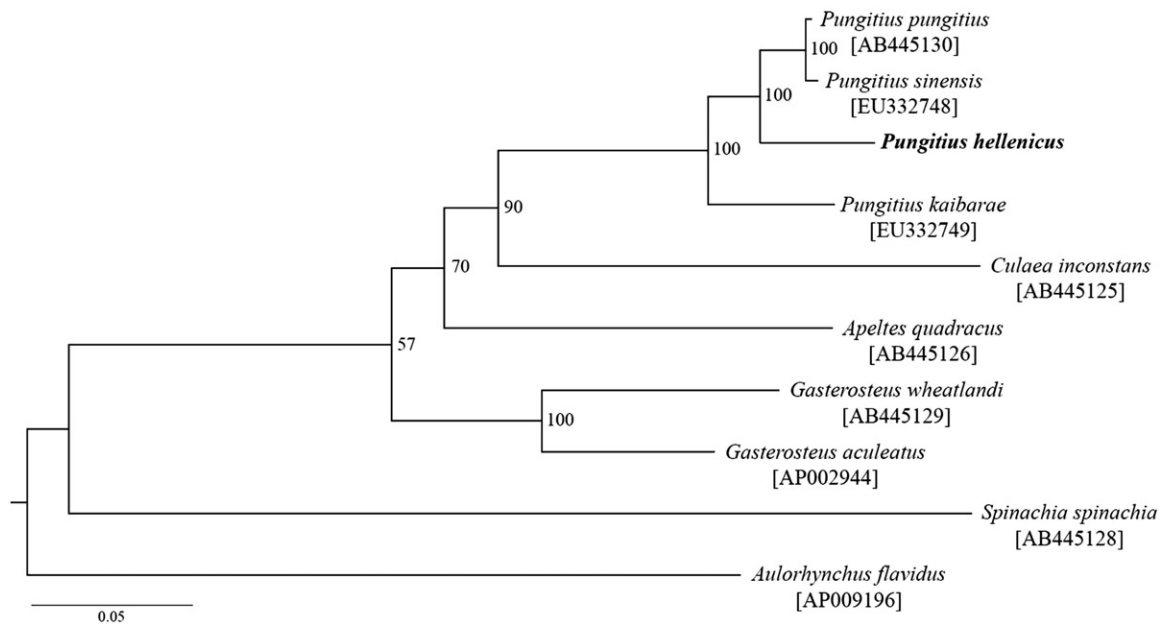


Figure 1. A maximum likelihood tree inferred from 37 mitochondrial genes among nine Gasterosteidae and an outgroup (*Aulorhynchus flavidus*) species. Bootstrap support is indicated at nodes. GenBank accession numbers are indicated in brackets.

Acknowledgements

We thank Pekka Ellonen, Laura Häkkinen, Tiina Hannunen and Sami Karja for help with laboratory and bioinformatic work. The sequencing was conducted at Finnish Institute for Molecular Medicine.

Disclosure statement

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

Funding information

This study was supported by grants (108601 & 118673) from the Academy of Finland, the Ministry of Culture of the Czech Republic (DKRVO 2015/15, National Museum, 00023272) and by the institutional resources of the Ministry of Education, Youth and Sports of the Czech Republic.

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