



Mitochondrial DNA Part B Resources

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MITOGENOME ANNOUNCEMENT

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

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ABSTRACT

The complete mitochondrial genome of the Sakhalin nine-spined stickleback *Pungitius tymensis* was determined using Illumina paired-end sequencing of genomic DNA. The genome sequence was 16 481 bp in length, consisting of 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes and a control region. The content and arrangement of the genes were identical to those of other Gasterosteidae species. *P. tymensis* was phylogenetically positioned with other *Pungitius* species (*P. kaibarae*, *P. pungitius* and *P. sinensis*) with a clear distinction from them. Nucleotide identity in the 37 genic regions ranged from 94.7% to 94.9% between *P. tymensis* and the other *Pungitius* species.

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Gasterosteidae; genome;
mtDNA; *Pungitius tymensis*

The teleost fishes in the genus *Pungitius* have become popular model species in ecology and evolutionary biology (Merilä 2013). Although their phylogenetic positioning and affinities relative to other genera within the Gasterosteidae are now well established (Kawahara et al. 2009), the phylogenetic relationships and taxonomic statuses of many

Pungitius species are still debated (Keivany & Nelson 2000; Mattern 2007). One of the enigmatic species, sometimes also recognized as a subspecies of *P. pungitius* (Keivany & Nelson 2000), is the Sakhalin nine-spined stickleback, *P. tymensis*. Recent studies based on partial mitochondrial DNA (mtDNA) fragments support its deep phylogenetic

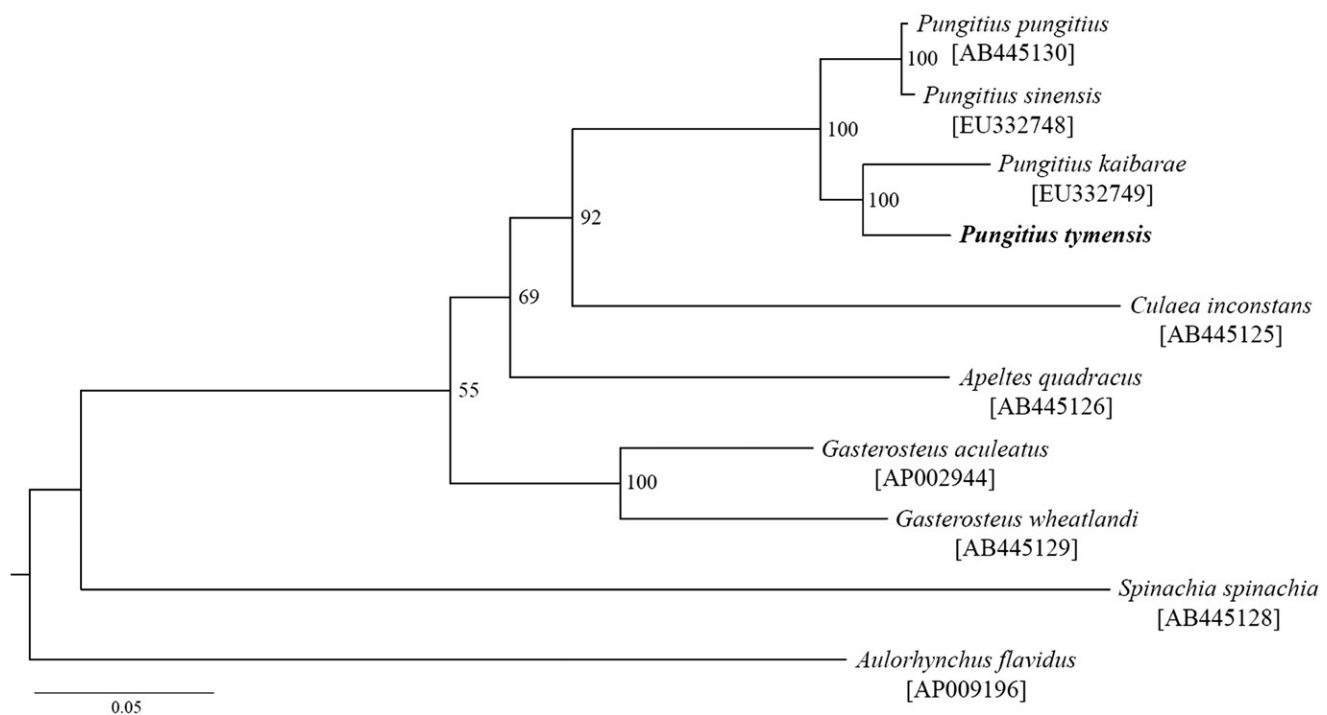


Figure 1. A maximum-likelihood tree inferred from 37 mitochondrial genes among nine Gasterosteidae and an outgroup (*Aulorhynchus flavidus*) species. Bootstrap support is indicated at nodes. GenBank accession numbers are indicated in brackets.

divergence from other *Pungitius* fishes (Bae & Suk 2015; Wang et al. 2015), but further insights to its evolutionary history could be gained with access to its complete mitochondrial genome.

P. tymensis was collected from Hokkaido Island, Japan (43°50'N, 145°05'E). A total of 9.7 million reads were generated from the genomic DNA using the Illumina HiSeq2000 platform with 100 paired-end strategy and aligned against the *P. sinensis* mitochondrial genome (Hwang et al. 2012a) using bwa-0.5.10 (Li & Durbin 2009). Mean sequence coverage across the mitochondrial genome was 40-fold, and all parts of the genome, except for two short regions (15 and 27 bp), had at least one-fold coverage (72.5% with ≥ 20 -fold coverage). To fill sequence gaps in the two regions, direct sequencing was performed for polymerase chain reaction (PCR) products obtained using two primer sets (L-Thr [Takahashi & Goto 2001] and PPCR607 [Wang et al. 2015]; PNADH-F, 5'-GTTGCTTCTTAACACTTCTTG-3' and PNADH-R, 5'-AGTTGGTCGTATCGAAATCG-3'), according to the procedures described in Teacher et al. (2011). The consensus sequence of the *P. tymensis* mitochondrial genome was constructed using SAMtools 1.2 (Li et al. 2009) with manual checking.

The complete mitochondrial genome of *P. tymensis* is 16 481 bp in length (GenBank Accession No. KU255082, including 13 protein-coding genes, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes and a control region. The content and arrangement of the genes were identical to those of other Gasterosteidae species (Miya et al. 2001; Kawahara et al. 2009; Hwang et al. 2012a,b). An incomplete stop codon was observed in four (*ND2*, *COII*, *ND4* and *Cytb*) out of the 13 protein-coding genes. The overall base composition of the entire mitochondrial genome was 27.2% for A, 26.4% for T, 17.6% for G and 28.8% for C. The phylogenetic position of *P. tymensis* in Gasterosteidae fishes was investigated based on the 37 genes (15 582 bp in total) with a maximum-likelihood approach using RAXML v.8.0 (under the GTR + GAMMA model, 37 gene partitions and 100 thorough bootstrap replicates; Stamatakis 2014). *P. tymensis* was phylogenetically positioned together with other *Pungitius* species (i.e., *P. kaibarae*, *P. pungitius* and *P. sinensis*) with a clear phylogenetic distinction from them (Figure 1). Nucleotide identity across the 37 genic regions ranged from 94.7% to 94.9% between *P. tymensis* and the other *Pungitius* species.

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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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