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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genome of a self-fertilizing fish *Kryptolebias marmoratus* (Cyprinodontiformes, Rivulidae) from Florida

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ABSTRACT

The complete mitochondrial genome was sequenced in a mangrove rivulus *Kryptolebias marmoratus* from western Florida using next-generation sequencing. The 17 329 bp-long genome was identical in length and 99.8% similar to a previously published genome of this species from a specimen of unknown geographic origin. Gene arrangement in *K. marmoratus* is similar to other cyprinodontiform fishes, except for the presence of a second copy of the control region inserted upstream of the *nad1* gene.

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Mangrove rivulus *Kryptolebias marmoratus* (Poey, 1880) is one of the only two known self-fertilizing hermaphroditic vertebrates (Avise & Tatarenkov, 2015). This small fish inhabits mangrove habitats along the tropical and subtropical western Atlantic basin. The geographic distribution of *K. marmoratus* was thought to be the widest among rivulid species, stretching from southeastern Brazil to central Florida (Tatarenkov et al., 2011), but recent taxonomic reorganizations rendered the geographical ranges of constituent species of the *marmoratus* complex uncertain. Only one complete mitochondrial (mt) genome of *K. marmoratus* is available (AF283503; Lee et al., 2001), which derives from a specimen of unknown geographic origin. The availability of additional genomes would help in choosing most informative mt regions for comprehensive phylogeographic studies, which are still absent for this unique fish.

We have sequenced the complete mt genome of *K. marmoratus* isolate FDS08 (GenBank accession no. KT893707) from near Fort de Soto Park in western Florida (N27°37'42.1", W082°42'13.6") using next-generation sequencing. A whole genome library was constructed with the Bioo Scientific NEXTflex kit (Bioo Scientific Corporation, Austin, TX) and sequenced in one lane of an Illumina HiSeq2500 sequencing system (Illumina Inc., San Deigo, CA) using paired-end 100 cycles chemistry. The mt genome was assembled in Geneious ver. 8.1.6 (Biomatters Ltd., Auckland, New Zealand) using AF283503 as a reference. The size of the assembled genome is 17 329 bp, which is identical to the one previously published (AF283503). The gene arrangement is also the same, after accounting for an incorrect annotation: a 69-bp region spanning positions 4724–4792 encodes tRNA-MET, but is mistakenly labeled as tRNA-Trp in AF283503. We note that the mistake affects only the GenBank accession, whereas

annotation is correct in the article announcing that sequence (Lee et al., 2001). There are 39 single-nucleotide differences between our sequence and AF283503, amounting to the sequence divergence of 0.0023 ± 0.0002 . The highest divergence is observed in *atp6* and the control region 1 (CR1 or D-loop) with six and five nucleotide differences, respectively. Whereas the D-loop is a commonly used locus in phylogeographic studies of fishes due to its high variability, *atp6* is less popular due to a presumed lack of variation. *cytB*, another locus popular in population genetics of fishes, showed rather little variation (only two nucleotide differences).

The D-loop is known to be duplicated in *K. marmoratus*, resulting in a second “control region” upstream of the *nad1* gene (Lee et al., 2001), with the 735-bp-long segments being highly similar between the two regions. In our mt genome, these segments (CR1: 16 448–17 182; CR2: 2813–3547) were identical, once again demonstrating concerted evolution of these paralogous regions, probably mediated by gene conversion (Tatarenkov & Avise, 2007).

We conducted a comparison of the mt genomes of all cyprinodontiform fishes available in GenBank as of October 6, 2015 and found that, with a few exceptions described below, the gene order is conserved. Altogether, there were 29 complete mt genomes representing 20 described species from seven cyprinodontiform families (Rivulidae, Nothobranchiidae, Aplocheilidae, Goodeidae, Cyprinodontidae, Poeciliidae, and Fundulidae), as shown in Figure 1. Apart from the mentioned duplication of the CR region in *K. marmoratus*, there are only two other species with re-arrangements. One species is *Xenotoca eiseni* (AP006777), in which tRNA-Met is duplicated and the second copy is inserted upstream of tRNA-Ile. In another species, *Nothobranchius furzeri* (EU650204), the 3' end of the mtDNA is modified considerably, involving duplication of tRNA-Glu with

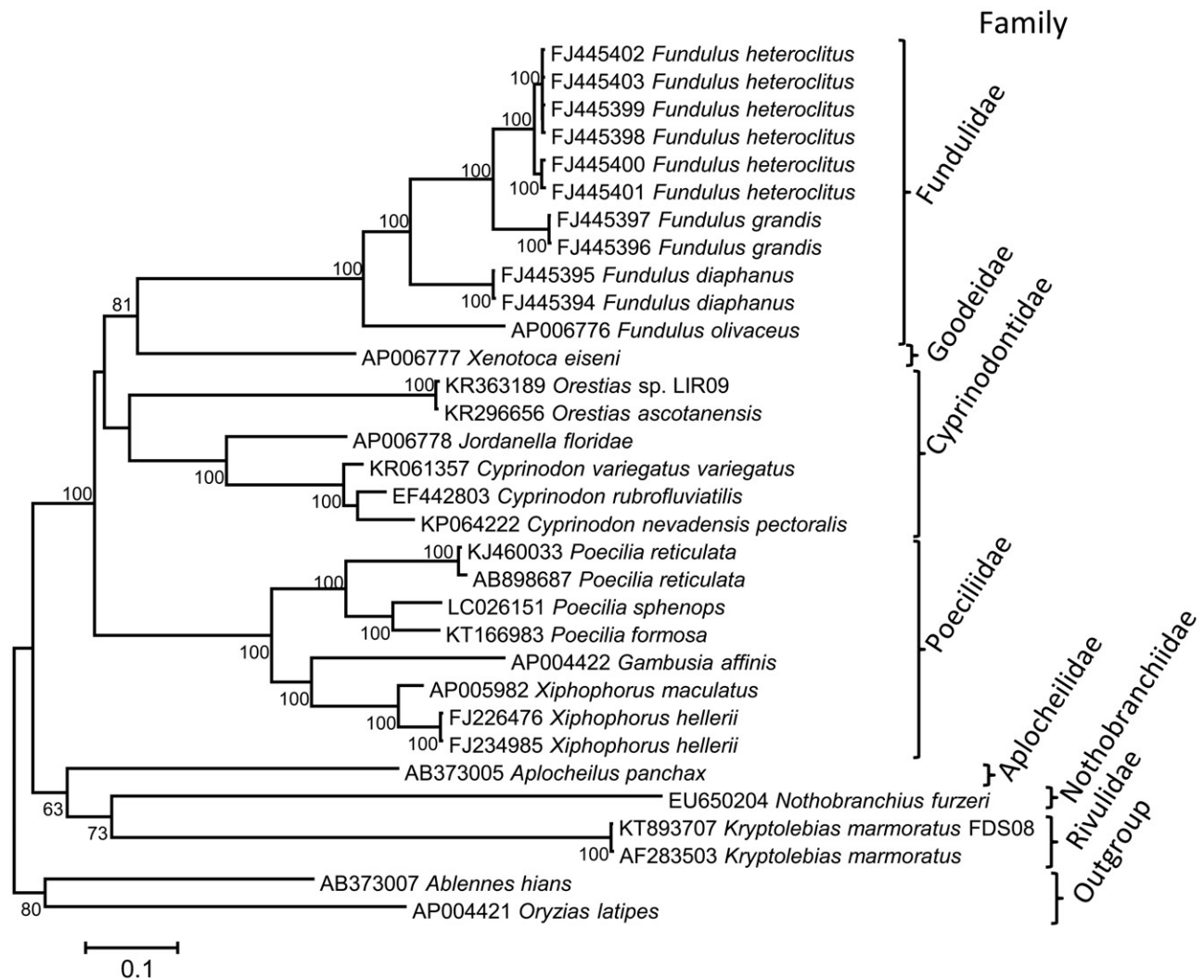


Figure 1. Maximum likelihood (ML) tree based on 13 protein-coding genes (11 326 bp) for the *K. marmoratus* isolate FDS08 and 29 GenBank representatives of the order Cyprinodontiformes with complete mt genomes. The tree is inferred with the Hasegawa–Kishino–Yano + gamma + invariant sites (HKY + G + I) model of nucleotide substitutions. The bootstrap support values (>50%) based on 500 replications are shown above the nodes. The outgroup is represented by two species from the order Beloniformes. A ML tree based on the whole mt genomic comparisons (not shown) has identical topology and similar bootstrap support values.

insertion of the second copy downstream of the D-loop and the original locus degrading into a pseudogene (this pseudogene is incorrectly classified as *tRNA-Gln* in EU650204). Furthermore, the D-loop of *N. furzeri* is disrupted by multiple insertions so that *tRNA-Thr* and *tRNA-Pro* are separated by 2091 bp, whereas these genes are adjacent in the other cyprinodontiforms.

The phylogenetic position of *K. marmoratus* (Figure 1) is in broad agreement with current understanding of the systematics of Cyprinodontiformes (Avisé & Tatarenkov, 2015; Pohl et al., 2015).

Declaration of interest

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The study was supported by funds from the University of California at Irvine.

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