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Research paper

# Characterization and phylogenetic analysis of complete mitochondrial genomes for two desert cyprinodontoid fishes, *Empetrichthys latos* and *Crenichthys baileyi*

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ABSTRACT

The Pahrump poolfish (*Empetrichthys latos*) and White River springfish (*Crenichthys baileyi*) are small-bodied teleost fishes (order Cyprinodontiformes) endemic to the arid Great Basin and Mojave Desert regions of western North America. These taxa survive as small, isolated populations in remote streams and springs and evolved to tolerate extreme conditions of high temperature and low dissolved oxygen. Both species have experienced severe population declines over the last 50–60 years that led to some subspecies being categorized with protected status under the U.S. Endangered Species Act. Here we report the first sequencing of the complete mitochondrial DNA genomes for both *E.* l. *latos* and the *moapae* subspecies of *C. baileyi*. Complete mitogenomes of 16,546 bp nucleotides were obtained from two *E.* l. *latos* individuals collected from introduced populations at Spring Mountain Ranch State Park and Shoshone Ponds Natural Area, Nevada, USA, while a single mitogenome of 16,537 bp was sequenced for *C. b. moapae*. The mitogenomes of both species contain 13 protein-encoding genes, twenty-two tRNAs, and two rRNAs (12S and 18S) following the syntenic arrangement typical of Actinopterygiian fish mitogenomes, as well as D-loop control regions of 858 bp for *E. latos* and 842 bp for *C. baileyi moapae*. The two *E. latos* individuals exhibited only 0.0181% nucleotide sequence divergence across the entire mitogenome, implying little intraspecific mtDNA genetic variation. Comparative phylogenetic analysis of the poolfish and springfish mitochondrial genomes to available mitogenomes of other Cyprinodontoid fishes confirmed the close relationship of these oviparous *Empetrichthys* and *Crenichthys* genera to the viviparous goodeid fishes of central Mexico, and showed the combined clade of these fishes to be a sister group to the Profundulidae killifishes. Despite several significant life history and morphological differences between the Empetrichthyinae and Goodienae, estimates of evolutionary genetic distances using two partial regions of mtDNA point to inclusion of the *Empetrichthys* and *Crenichthys* genera within the family Goodeidae along with the goodeid fishes of central Mexico.

## 1. Introduction

The native fish fauna of the arid North American west includes several taxa of small-bodied killifishes (order Cyprinodontiformes) that evolved within the harsh conditions of aquatic habitats within the Great Basin and Mojave Desert regions of the United States (Miller, 1948, 1950; Minckley and Marsh, 2009; Minckley and Deacon, 1968). Endemic to this region are the Pahrump poolfish (*Empetrichthys latos*) and the White River springfish (*Crenichthys baileyi*), two species that have experienced severe population declines over the past ~50 years due to the combined influences of habitat loss, habitat degradation, and invasive species (Deacon and Williams, 2010; Miller et al., 1989; Williams et al., 1985). Despite extensive study of the phylogenetic organization of fishes within the order Cyprinodontiformes (e.g., Breden et al., 1999; Echelle, 2008; Martin et al., 2016; Meredith et al., 2010; Kang et al., 2013), there remains uncertainty about the taxonomic classification of the *Empetrichthys* and *Crenichthys* genera within the Cyprinodontiformes (e.g., Grant and Riddle, 1995). These

*Abbreviations:* ATP6 and ATP8, ATPase subunits 6 and 8; bp, Base pairs; CO1–CO3, Cytochrome c oxidase subunits I–III; ND1–ND6 and ND4L, NADH dehydrogenase subunits 1–6 and 4L; rRNA, Ribosomal RNA; tRNA, Transfer RNA

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genera have sometimes been grouped as their own family Empetrichthyidae (Miller and Smith, 1986; Miller et al., 2005; Minckley and Marsh, 2009) or alternatively been assigned to subfamily Empetrichthyinae within family Goodeidae (La Rivers, 1994; Parenti, 1981; Webb, 1998).

The Pahrump poolfish (*Empetrichthys latos*) is the sole remaining taxon of the genus *Empetrichthys* since its sister species – the Ash Meadows killifish *E. merriami* (Gilbert, 1893; Jordan and Evermann, 1896) – went extinct in the early 1950's (Miller et al., 1989; Pister, 1990; Soltz and Naiman, 1978). The only other known member of the genus is the extinct *Empetrichthys erdisi*, described from a fossil specimen discovered in southern California, USA, from Pliocene Colorado River deposits (Uyeno and Miller, 1962). Miller (1948) first characterized *E. latos* as a distinct species comprised of three morphologically-distinct subspecies that occurred in isolated groundwater-fed springs in the Pahrump Valley, Nevada, USA. The combined effects of physical habitat alteration, groundwater withdrawal that reduced surface water flow, and the introduction of non-native species contributed to the extinction of two of these subspecies (*E. l. pahrump* and *E. l. concavus*) (Deacon and Williams, 2010; Miller et al., 1989; Minckley et al., 1991). The only remaining subspecies, *E. l. latos*, was endemic to Manse Spring in Pahrump Valley, Nevada. Population surveys indicated that this population underwent dramatic declines to fewer than 50 fish in 1962–63 and again in 1967–68 (Deacon and Williams, 2010). The precarious status of this species led to its listing as 'endangered' in 1967 under the U.S. Endangered Species Preservation Act. Individuals of this sole remaining taxon of *E. latos* were translocated to refuge habitats in the 1970s prior to the complete failure of groundwater flow of Manse Spring, in 1975 (Deacon and Williams, 2010; Goodchild, 2016; Minckley et al., 1991). Since the extirpation from its native range, *E. latos* persists to this day entirely through ongoing active management of translocated refuge populations in Nevada, USA.

*E. latos* from Manse Spring were originally transplanted into three fishless locations in Nevada during the early 1970s: Los Latos Pools near Lake Mohave in the Lake Mead National Recreation Area, Nevada, the U.S. Bureau of Land Management Shoshone Ponds Natural Area southeast of Ely, Nevada, and Corn Creek on the Desert National Wildlife Refuge, Nevada (Fig. 1). The refuge populations at Los Latos Pools and Shoshone Ponds later failed in ~1977 and 1974, respectively (U.S. Fish and Wildlife Service, 2004), and all extant refuge populations of Pahrump poolfish are descended from the population established with 29 poolfish at Corn Creek in 1971. In 1976, 66 poolfish were translocated from Corn Creek to re-establish a refuge population at the Shoshone Ponds Natural Area, where poolfish have been actively managed across a number of small ponds and a small stream since that time. In 1983, 426 poolfish were used to establish a refuge population Lake Harriet in Spring Mountain Ranch State Park west of Las Vegas, Nevada (Fig. 1; also see Goodchild, 2016). To date, however, the Pahrump poolfish continues to remain vulnerable to demographic effects, genetic drift, non-native species, and natural catastrophes (Deacon and Williams, 2010; Goodchild and Stockwell, 2016), as evidenced by the recent (2015–2016) compromise of the Lake Harriet population by invasive species (U.S. Fish and Wildlife Service, 2016).

The White River springfish (*Crenichthys baileyi*) has also experienced severe declines in range and numbers. Since its original mistaken description as a subspecies of the desert pupfish *Cyprinodon macularius* in 1893 (Gilbert, 1893) and later redesignation as a separate species of *Cyprinodon* (Jordan and Evermann, 1896), it was subsequently reassigned to the new genus *Crenichthys* (Hubbs, 1932) by Sumner and Sargent (1940). Similar to many fishes of the arid southwest of North America, the White River springfish is capable of surviving low oxygen, high temperature conditions (Hubbs and Hettler, 1964; Sumner and Sargent, 1940; Sumner and Lanham, 1942), and populations of this springfish are found only in several warm water springs and their outflows scattered within the pluvial White River system of southeastern Nevada. Five subspecies of *C. baileyi* have been described based on morphological differentiation (Williams and Wilde, 1981; see also Minckley and Marsh, 2009), and *C. baileyi* has only one extant congener, the Railroad Valley springfish (*C. nevadae*), that occurs in isolated warm water springs in the Railroad Valley of Nevada (Hubbs, 1932). Physical environmental degradation and non-native species has led to severe population declines and listing of the *C. b. baileyi* and *C. b. grandis* subspecies as 'endangered' under the U.S. Endangered Species Act. The *moapae* subspecies occurs in several warm headwater springs (~31–32 °C at the spring sources) and their outflows in the upper Muddy River (Deacon and Bradley, 1972; Scoppettone et al., 1998). The introduction of nonnative species to these habitats has led to declines in the size of *C. b. moapae* populations (Cross, 1976; Scoppettone, 1993), although recent conservation efforts (i.e., habitat restoration) to sustain populations of the cohabitating Moapa dace (*Moapa coriacea*) have also enhanced *C. b. moapae* populations in some of these habitats (e.g., Syzdek, 2013).

In this study, we sequenced the complete mitochondrial genomes of two *E.* l. *latos* individuals, one each from Shoshone Ponds and from Lake Harriet at Spring Mountain Ranch. We also sequenced the full mitogenome of a single *C. b. moapae*. For each fish, we describe the nucleotide composition of the complete mitogenome and identified the genome organization, gene order and codon usage. We also analyzed the molecular phylogenetic relationships of these *Empetrichthys* and *Crenichthys* fishes relative to other cyprinodontids in an effort to clarify the taxonomic relationships and status of these genera within the order Cyprinodontiformes.

## 2. Materials and methods

### 2.1. Specimen collection

Individual *E.* l. *latos* were collected from two populations: Spring Mountain Ranch State Park (36° 4′5.16″N, 115°27′41.92″W; Clark County, Nevada, USA) on 23 August 2013, and Shoshone Ponds Natural Area (38°56′22.43″N, 114°25′4.36″W; White Pine County, Nevada, USA) on 8 August 2012. In addition, a single *C. baileyi moapae* individual was collected from Plummer Stream in the upper Muddy River complex (36°42′39.03″N, 114°42′42.81″W; Clark County, Nevada, USA) on 19 August 2012. Tissues from all specimens were stored in 95% EtOH.

### 2.2. Primer design, mitogenome amplification, and sequencing

Genomic DNA was isolated from the skeletal muscle of each specimen using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), and then quantified using a P300 NanoPhotometer (Implen, Inc.). Overlapping regions of the mtDNA genome for each species were amplified in PCR reactions containing 25 μL of 2 × GoTaq® Long PCR Master Mix (Promega Corp., Madison, WI, USA), 1 μL each of forward and reverse primer (10 mM), 13 to 18 μL of nuclease-free $H_2O$, and 5 to 10 μL of DNA template (85 to 298 ng·mL$^{-1}$). Multiple pairs of oligo primers were designed to partial mtDNA sequences for cytochrome *b* (*Cyt B*), cytochrome *c* oxidase subunit-I (*COI*), or the D-loop region available for each species on GenBank (http://www.ncbi.nlm.nih.gov/) (*E. latos*: **U09108**, **AY356573**, **AY356615**; *C. baileyi*: **U09102**, **AF510819**, **AY356571**, **AY356614**) to generate overlapping PCR products that spanned the entire mitogenomes of both taxa. Nucleotide sequences for these primers are provided in Table 1. All PCR products were examined on 0.8% ethidium bromide gels, and products with bands of expected size were cleaned (QIAquick PCR Purification Kit; Qiagen) before being Sanger sequenced with the same primers used for PCR (Molecular Cloning Laboratories, South San Francisco, CA, USA). The initial partial mtDNA sequences obtained were then used to design additional gene-specific primers (see Supplementary materials, Tables S1 and S2) that were used to Sanger sequence the complete mitogenomes of both species by primer walking.