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ARE THE ENDANGERED SPRINGFISH (*CRENICHTHYS* HUBBS) AND POOLFISH (*EMPETRICHTHYS* GILBERT) FUNDULINES OR GOODEIDS?: A MITOCHONDRIAL DNA ASSESSMENT.—Two cyprinodontiform fish genera described by Gilbert (1893) comprise a total of four species (*Crenichthys baileyi* Gilbert, *Crenichthys nevadae* Hubbs, *Empetrichthys latos* Miller, and *Empetrichthys merriami* Gilbert) that are or were restricted to small, isolated pools and streams in the Great Basin and Mojave deserts of Nevada (Fig. 1). The two genera are diagnosed from one another by tooth morphology, pharyngeal teeth, fin morphology, and feeding habits (Parenti, 1981). Because of relictual distribution and susceptibility to habitat perturbation, *E. merriami* is now extinct (Minckley and Deacon, 1968) and *E. latos* remains only in a population translocated outside of its native range (Soltz and Naiman, 1978). The taxonomy of *Crenichthys* and *Empetrichthys* has been unstable and controversial over the past century. A postulated sister-group relationship of the two genera is supported by morphological features of the anal radials, maxillaries, premaxilla, and articular (Parenti, 1981). Prior to revision of cyprinodontiforms by Parenti (1981), the two genera have been aligned with Cyprinodontidae (Gilbert, 1893), Poeciliidae (Gill, 1894), Fundulininae (Hubbs, 1932), or placed in their own family, Empetrichthyidae (Jordan et al., 1930; Miller and Smith, 1986). Parenti (1981) presented a novel hypothesis by placing *Crenichthys* and *Empetrichthys* within the family Goodeidae, making them geographically disjunct, oviparous members of an otherwise viviparous family restricted to central Mexico (Fig. 1). Miller and Smith (1986) placed *Crenichthys* and *Empetrichthys* within Empetrichthyidae but concurred with Parenti (1981) relative to the sister relationship of this family to Goodeidae. Based on the latest revision of cyprinodontiform phylogeny (Parenti, 1981), the competing hypotheses of phylogenetic affinities of *Crenichthys* and *Empetrichthys* are shown in Figure 2A: one hypothesis is that the two genera are aligned with taxa in Parenti's funduline lineage (Hubbs, 1932; Rosen, 1964); the other hypothesis is that they belong to the goodeid lineage (Parenti, 1981; Miller and Smith, 1986). In this study, we use nucleotide sequence data

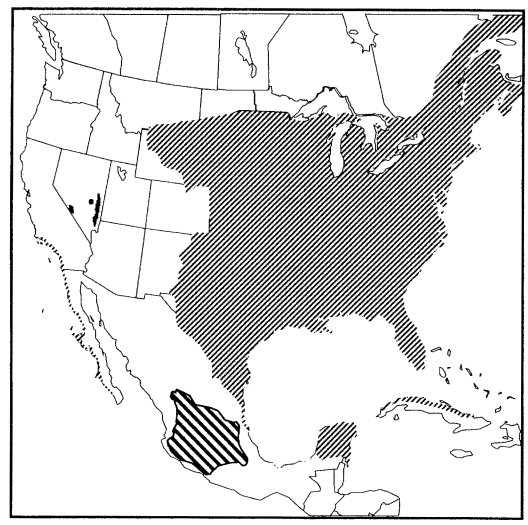

Fig. 1. Distribution of lineages in this study (from Parenti, 1981). Dark shading = *Crenichthys* and *Empetrichthys*; Light shading = fundulines; Cross-hatched = goodeids.

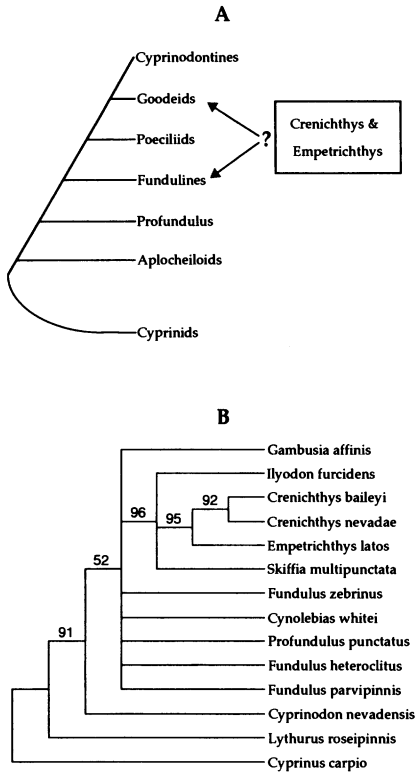

Fig. 2. (A) Partial phylogeny of cyprinodontiforms based on Parenti (1981), showing alternative hypotheses for placement of *Crenichthys* and *Empetrichthys*; (B) shortest parsimony tree using weighting strategy 3 (generalized parsimony at first and second base positions; transversion parsimony at third base positions). Numbers above branches indicate percentage of 500 bootstrap replicates that support each node. Only nodes receiving 50% or greater bootstrap support are shown as fully resolved.

from mitochondrial (mt)DNA to test these hypotheses.

Methods.—A total of 14 individuals were examined in this study, representing terminal taxa in a reduced phylogeny of cyprinodontiformes (Fig. 2A) based on Parenti (1981). Genomic DNA was isolated according to the protocol of Hillis et al. (1990). Primers published elsewhere (Smith and Patton, 1991) were used to PCR-amplify a region of the mtDNA cytochrome *b* gene according to published protocols (Allard et al., 1991; Riddle et al., 1993). A total of 314 base pairs (bps) of sequence were aligned by eye, with the first base corresponding to position 14,787 of human mtDNA (Anderson et al., 1981). Nucleotide sequences were translated to amino acid sequences using MacVector v3.5 for verification of reading frame integrity. After excluding sites with missing data, sequence divergence among samples was calculated using a maximum likelihood approach (DNADIST in PHYLIP v3.5; Felsenstein, 1993), with the "T" option of 10 to partially correct for bias in transition vs transversion substitution rates (Thomas and Beckenbach, 1989). Phylogenetic relationships were inferred from distance matrices by using the neighbor-joining approach (Saitou and Nei, 1987). Nucleotide sequences also were used as discrete characters in a maximum-parsimony analysis that employed PAUP v3.0 (Swofford, 1991) and majority-rule consensus to produce a shortest consensus tree. Bootstrapping (500 replications) was used to evaluate support for branches of the consensus tree. A growing body of evidence suggests that the ability of parsimony analysis to produce the correct phylogeny is influenced by the relative weights given to subsets of characters in the analysis, and giving greater weight to more slowly evolving characters may improve performance of the algorithm if rates of change are high (Huelsenbeck and Hillis, 1993). We conducted analyses using three weighting strategies: (1) generalized parsimony; (2) exclusion of third codon position characters; and (3) a mixed weighting approach with generalized parsimony of first and second position and transversion parsimony of third position characters. Trees were rooted with two outgroup taxa: *Lythrurus roseipinnis* (Schmidt and Gold, 1993) and *Cyprinus carpio* (Araya et al., 1984).

Results.—Pairwise sequence divergence values pertinent to hypotheses addressed in this study follow (number of aligned bases in parentheses): *C. baileyi–C. nevadae* (284) = 3.4%; *Crenichthys–Empetrichthys* (284) = 8.8%; *Crenichthys / Empetrichthys–Ilyodon / Skiffia* (263/232) = avg. 20.7%; *Crenichthys / Empetrichthys–Fundulus* spp. (242) = avg. 35.1%; *Crenichthys / Empetrichthys–Profundulus* (285) = 29.5%; *Crenichthys / Empetrichthys–Gambusia* (287) = 28.3%; *Crenichthys / Empetrichthys–Cyprinodon* (275) = 31.0%; and *Crenichthys / Empetrichthys–Cynolebias* (284) = 39.4%. Maximum-parsimony analyses were based on 121 phylogenetically informative base positions. The consensus tree resulting from weighting strategy 3, shown in Figure 2B, was 207 steps long. Trees that placed *Crenichthys* and *Empetrichthys* with either *F. zebrinus*, *F. heteroclitus*, or *F. parvipinnis* required an additional 11, 12, or 16 steps, respectively. The hypothesis of relationships {[(*C. baileyi*, *C. nevadae*) *E. latos*] *I. furcidens*—*S. multipunctatus*} also received strong (73% and 92%, respectively) bootstrap support in parsimony analyses using weighting strate-

gies 1 and 2 and in a neighbor-joining tree using a distance matrix generated from first and second codon position characters.

Discussion.—Results of this study support a sister-group relationship between *Crenichthys* and *Empetrichthys* and their phylogenetic affinity with the family Goodeidae rather than with representative fundulines, poeciliids, or cyprinodontines. Our analyses do not provide a clear hypothesis of branching order for *Crenichthys* + *Empetrichthys* relative to the two putative members of the Goodeidae used in this study. Fossils assignable to *Empetrichthys* have been recovered from Miocene deposits in southern California (Uyeno and Miller, 1962), providing an estimated minimum age for this taxon of five million years. The northern displacement of *Crenichthys* + *Empetrichthys* is compatible with several models of biogeographic history, including a formerly widespread distribution followed by extinction in intervening areas (Parenti, 1981). Alternatively, this may represent disjunction resulting from northward displacement of a formerly more southern terrane through mid-Tertiary tectonic activity (Sedlock et al., 1993). Clearly, *Crenichthys* and *Empetrichthys* represent a biogeographically and biologically interesting lineage of cyprinodontiform fishes.

Material examined.—Information listed for each specimen includes species, collection locality, catalog number, and GENBANK accession number. All voucher specimens are housed at the University of Nevada, Las Vegas, unless otherwise noted. *Cyprinodon nevadensis*: Amargosa River south of Tecopa, Inyo County, California, LVT 743, U 09107. *Fundulus zebrinus*: tributary of Pecos River, Eddy County, New Mexico, LVT 430, U 09109. *Ilyodon furcidens*: aquarium stock, Rio del Tule, Colima, Mexico, LVT 1745, U 09111. *Skiffia multipunctata*: aquarium stock, Rio Santa Catarina, Michoacan, Mexico, LVT 1743, U 09113. *Gambusia affinis*: Flamingo Wash, Las Vegas, Clark County, Nevada, LVT 1734, U 09110. *Profundulus punctatus*: SE LaRaforma, Oaxaca, Mexico, LVT 1748, U 09112. *Cynolebias whitei*: aquarium stock, near Rio de Janeiro, Brazil, LVT 1739, U 09130. *Crenichthys baileyi*: Hiko Spring, Lincoln County, Nevada, LVT 1749, U 09102. *Crenichthys nevadae*: Big Spring, Nye County, Nevada, LVT 1759, U 09105. *Empetrichthys latos*: Corn Creek, Clark County, Nevada, LVT 1763, U 09108. GENBANK accession numbers for sequences of *Fundulus heteroclitus* and *Fundulus parvipinnis* (provided by G. Bernardi) are L 23771 and L 23773, respectively.

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FEMALE CHOICE IN *BUFO AMERICANUS*: EFFECTS OF DOMINANT FREQUENCY AND CALL ORDER.—Vocalizations by male anurans can influence both interactions between males and mate choice by females (Gerhardt, 1988; Wells, 1988; Ryan, 1991). Vocal interactions between neighboring males in a chorus can affect call timing (alternation, overlap, or synchrony), rate, complexity (number of similar or distinct notes), dominant frequency, and type (advertisement or aggressive) as well as the likelihood of physical combat (Wells, 1988; Wagner, 1989). Female mating preferences can be influenced by call parameters such as rate (number of calls/min), duration, pulse rate, and dominant frequency (Gerhardt, 1988). Interactions between temporal and spectral parameters such as call timing and dominant frequency, respectively, can also affect male-male and male-female behaviors (Dyson and Passmore, 1988).

We present results of an experiment on the effect of temporal overlap in male advertisement calls on female preference when alternative calls differ in dominant frequency. Male advertisement calls in the study organism, *Bufo americanus*, are long trills, averaging 8 sec in our study populations (range: 1.1–18.6 sec), and temporal overlap commonly occurs in calls of adjacent chorusing males. We also provide data on the effect of female age, body size, and prior breeding experience on call preference patterns.

Bufo americanus typically breed for a brief period in late spring (Howard, 1988). Previous studies on the populations used in this study during 1985–1992 (Howard, 1988; Howard et al., In Press) have revealed that larger males often have a mating advantage and that females usually select larger than average males when they initiate pair formation. Similar nonrandom mating patterns have been found in some populations of *B. americanus* (Licht, 1976; Gatz, 1981) but not in others (Kruse, 1981; Sullivan, 1992). Criteria used by females to ascertain male size in our study populations are unknown but are presumed to include at least some vocal characteristics. Male size influences the dominant frequency of calls in many species (Forester and Czarnowsky, 1985; Robertson, 1986; Ryan, 1980) including *B. americanus* (R. D. Howard and J. R. Young, unpubl.; Sullivan, 1992, for one of two years sampled) and call intensity as measured by sound pressure level (SPL; Gerhardt, 1975). Temporal call parameters such as call rate and call duration are size dependent in some species (Greene, 1990) but not in other species (Sullivan, 1982, 1984) including *B. americanus* (Howard, 1988; Sullivan, 1992; R. D. Howard and J. R. Young, unpubl.). Based on these observations, we concentrated on dominant frequency as the spectral cue in male calls that females use to judge male size and predicted that females should prefer calls