The gill of the killifish, *Fundulus heteroclitus*, expresses two different EP_1 receptors

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Teleost gill tissue produces prostaglandins[1,2]. Partial cDNAs for COX-1 and COX-2 have been cloned and sequenced from the killifish gill[3,4], and PGE_2 inhibits active salt extrusion by the killifish opercular epithelium[5] (the generally accepted model for the marine teleost gill extrusion mechanisms). Thus, one might expect that teleost gill tissue should express E-type prostaglandin receptors[6]. Therefore, the goal of this study was to determine if an E-type prostaglandin receptor, specifically EP_1 or EP_3, is expressed in the gills of the euryhaline killifish, *Fundulus heteroclitus*.

Killifish were acquired at the Mount Desert Island Biological Lab (MDIBL), and gills were frozen in liquid nitrogen. Total RNA isolation and cDNA synthesis were as previously described[4]. Degenerate primers were designed to promote amplification of either EP_1 or EP_3. PCR, product visualization, cloning, sequencing and sequence analysis were as previously described[4]. PepTools software (BioTools, Inc.) was used to align predicted amino acid sequences with full-length EP sequences from other chordates. MEGA software[7] was used with a minimum evolution and bootstrap (500 replicates) analysis to generate a phylogenetic tree that included the novel EP1 sequences and other known EP sequences.

Two EP_1 orthologues, EP_{1a} and EP_{1b}, were cloned from the killifish gill. Degenerate primers supported the amplification of a 702 bp product that was found to be 74.8% identical to zebrafish (*Danio rerio*) EP_1 and a 714 bp product that was found to be 63.0% identical to zebrafish EP_1. These 234 and 238 amino acid sequences were aligned with other EP protein sequences to generate a phylogenetic tree (Figure 1). This is the first documented case of two EP_1 receptors in a teleost.

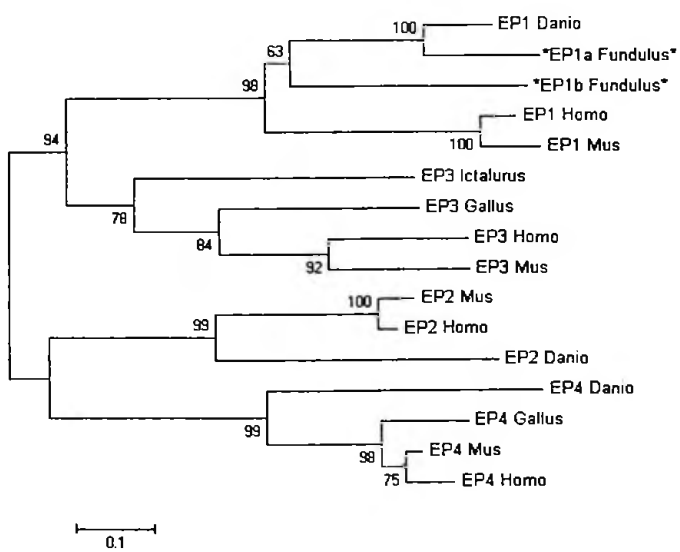

Figure 1. A phylogenetic analysis of cyclooxygenase sequences with our killifish sequences added (*denotes novel sequences). The amino acid sequences were aligned with Peptool (Biotools 2.0), and MEGA was used in a minimum evolution analysis with a bootstrap of 500 replicates to generate the phylogenetic tree. The scale bar represents 0.1 amino acid substitutions.

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