Cypress Creek is a northern tributary to the Tennessee River (Pickwick Lake) located in Lauderdale County, Alabama and Wayne County, Tennessee which drains an area of approximately 650 km2 in the Western Highland Rim Ecoregion. Although sampled for fishes over the past several decades at several localities, the last comprehensive fish survey of this watershed was conducted in 1971, and changes in the fish fauna may have occurred. Data from over 2,000 historical records was compiled and distribution maps were generated for every species known from Cypress Creek. Sampling was conducted in 2015–2016 by seining and backpack electrofishing and community composition was analyzed using the Index of Biotic Integrity (IBI). Collections yielded a total of 66 species, with a mean of 21 species per collection, including several species of conservation interest. The known fish fauna of Cypress Creek is now 102 species, which represents high species diversity for this moderate-sized stream system. IBI scores varied among major tributaries, suggesting that differing land uses across the watershed may impact fish community composition. Continuing development within the watershed should be closely monitored to insure the biodiversity of fishes and other aquatic organisms in Cypress Creek is not compromised.