Geographic barriers that limit the movement of individuals between populations may create or maintain phylogenetically discrete lineages. Such barriers are often inferred from geographic surveys of a single mitochondrial marker to identify phylogenetic splits. Mitochondrial DNA, however, has an effective population size one-fourth that of nuclear DNA, which can facilitate the rapid evolution of monophyletic mtDNA lineages in the absence of geographic barriers. The identification of geographic barriers will thus be more robust if barriers are proposed a priori, and tested with multiple independent genetic markers in multiple species. Here, we tested two proposed marine biogeographic breaks located at the Mona Passage in the Caribbean Sea and at the southern end of Exuma Sound in the Bahamas. We sequenced mitochondrial cytochrome b (400 bp) and nuclear rag1 (573 bp) for nine species and color forms (183 individuals total) within the teleost genus Elacatinus (Gobiidae) that span the proposed breaks. Our results showed that Mona Passage separated mtcyb and rag1 lineages, with no genetic exchange between populations separated by just 23 km. However, the Central Bahamas barrier was only weakly supported by our data. Importantly, neither barrier coincided with deep genetic splits. This suggests that these two barriers did not initially isolate regional populations, but instead disrupt ongoing gene flow between regions. Our inferred relationships further suggested a division of the Caribbean region into northwestern and southeastern regions, a pattern reflected by some freshwater and terrestrial vertebrates. Our results, coupled with genetic and demographic data from other reef fishes and corals, provide robust support for the Mona Passage as a long-term biogeographic barrier for Caribbean animals.