

## **Application of Exon Primed Intron Crossing Markers to Cross-Amplify Oreochromis Species in Eastern Africa**

Oreochromis species are of economic importance in fisheries and aquaculture but threatened by human-mediated activities. Effective conservation and their sustainable management require genetic monitoring tools that can assess genetic variation across species. Various neutral markers have been used to monitor biodiversity in organisms, but they are limited in cross-amplification among various taxa. Non-neutral markers such as Exon-Primed Intron Crossing (EPIC) not only cross amplify various taxa but also target gene regions that are likely to be involved in adaptive responses to selective pressure. This study therefore developed 50 EPIC markers from *Oreochromis niloticus* reference genome targeting immune related genes to assess their potential to cross-amplify *Oreochromis* species. Genetic diversity, population structure, and differentiation was measured among *Oreochromis niloticus*, *O. jipe*, *O. esculentus* and *O. leucostictus* species and their respective populations from eleven African water bodies and two aquaculture farms. 45 EPIC markers successfully amplified target regions across the four *Oreochromis* species. *O. niloticus* exhibited the highest diversity ( $H_e = 0.50$ ), followed by *O. jipe* ( $H_e = 0.29$ ), *O. leucostictus* ( $H_e = 0.28$ ) and *O. esculentus* with the lowest ( $H_e = 0.25$ ). The highest differentiation values were observed among populations of different species while the lowest was among farmed *O. niloticus*. This was supported by AMOVA results that showed highest genetic differentiation among species. Population structure analyses were concordant with species boundaries while showing detailed intraspecific clustering patterns among the *O. niloticus* populations that reflected geographical origin. The observed genetic diversity, gene flow, and population differentiation confirms the applicability of EPIC markers as molecular tools for monitoring biodiversity and guiding the conservation of *Oreochromis* species under increasing anthropogenic and environmental pressures.