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Molecular phylogenetics and divergence time estimation of cichlid fishes in African rift lakes

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Abstract

Why have cichlid fishes in the African Rift Lakes generated more species in less time than almost any other vertebrate lineage? Answering this demands reliable phylogenetic frameworks and accurate divergence time estimates. This research reconstructed molecular phylogenies for 42 cichlid species spanning Lakes Victoria, Malawi, and Tanganyika using concatenated mitochondrial (cytb, ND2) and nuclear (RAG1, S7) markers. Bayesian relaxed-clock analysis estimated divergence times using fossil-calibrated priors. Maximum likelihood and Bayesian inference recovered congruent topologies that placed Tanganyika lineages as basal, with the Victoria and Malawi clades forming a derived clade. Divergence of the Tanganyika tribes was dated to approximately 9.7 Mya, while the Victoria-Malawi split occurred around 4.8 Mya. The Victoria superclade showed an exceptionally recent radiation at roughly 0.15 Mya. These timelines reinforce the view that cichlid diversification accelerated dramatically in younger lakes and that ecological opportunity, rather than time alone, drives species proliferation.

Keywords: Molecular phylogenetics, divergence time estimation, cichlid fishes, African rift lakes, adaptive radiation, Bayesian inference, fossil calibration, speciation rate

Introduction

Few groups of organisms pose as sharp a challenge to evolutionary theory as the cichlid fishes of East Africa's Great Rift Lakes. Lake Victoria alone harbors over 500 species that appear to have arisen within the last 15,000 to 100,000 years—a pace of speciation that lacks a clear parallel among vertebrates ^[1]. Lakes Malawi and Tanganyika add hundreds more species, each lake with its own radiation shaped by distinct geological and ecological histories ^[2]. Molecular phylogenetics has become the primary tool for untangling these relationships, but early single-gene trees often produced conflicting topologies due to incomplete lineage sorting and hybridization ^[3]. Multi-locus approaches provide better resolution, and relaxed-clock methods allow divergence times to be estimated without assuming a constant rate of molecular evolution ^[4]. Yet broad comparative phylogenies that span all three major lakes with consistent marker sets and calibration strategies remain uncommon ^[5]. This research aimed to reconstruct a multi-gene phylogeny for 42 cichlid species representing major lineages from Lakes Victoria, Malawi, and Tanganyika, and to estimate divergence times using fossil-calibrated Bayesian methods.

Materials and Methods**Materials**

Molecular work was performed at the Department of Systematics and Evolution, Andaman Institute of Marine Biology, Port Blair, India, from January 2023 to December 2024. Tissue samples (fin clips preserved in 95% ethanol) for 42 cichlid species were obtained from museum collections at the Royal Museum for Central Africa (Tervuren, Belgium) and the Natural History Museum (London, UK) under material transfer agreements. DNA was extracted using a standard phenol-chloroform protocol. Species representation included 12 from Lake Victoria, 14 from Lake Malawi, and 16 from Lake Tanganyika, plus two outgroup taxa (*Tylochromis polylepis* and *Oreochromis niloticus*).

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Methods

Four gene regions were amplified: mitochondrial cytochrome b (cytb) and NADH dehydrogenase subunit 2 (ND2), and nuclear recombination activating gene 1 (RAG1) and ribosomal protein S7 intron 1. PCR products were Sanger sequenced bidirectionally. Sequences were aligned in MAFFT v7.4^[6] and trimmed to remove ambiguous ends. Partition schemes and substitution models were selected using PartitionFinder2^[7]. Maximum likelihood analysis was conducted in RAxML v8.2 with 1,000 bootstrap replicates^[8]. Bayesian inference used MrBayes v3.2 with two independent runs of 20 million generations^[9]. Divergence times were estimated in BEAST2 v2.6 using a relaxed lognormal clock and Yule speciation prior^[10]. Two fossil calibrations were applied: the oldest known cichlid fossil (*Mahengechromis*, 46 Mya) as a root minimum age, and a Tanganyika tribe fossil (8.5 Mya minimum) for internal calibration.

Results

Table 1: Divergence Time Estimates for Major Cichlid Clades

Clade/Split	Mean Age (Mya)	95% HPD (Mya)	PP
Root (Cichlidae)	48.3	44.1-53.7	1.00
Tanganyika basal	12.1	9.8-14.6	0.99
Tanganyika tribes	9.7	7.9-11.8	0.98
Victoria-Malawi split	4.8	3.6-6.2	0.97
Malawi flock	2.3	1.7-3.1	0.96
Victoria superflock	0.15	0.08-0.24	0.94

Bayesian and ML topologies were congruent at all well-supported nodes. Tanganyika lineages formed the basal radiation, with Victoria and Malawi species nested within a derived clade. The Victoria superflock showed the youngest divergence time (mean 0.15 Mya), consistent with a post-Pleistocene origin.

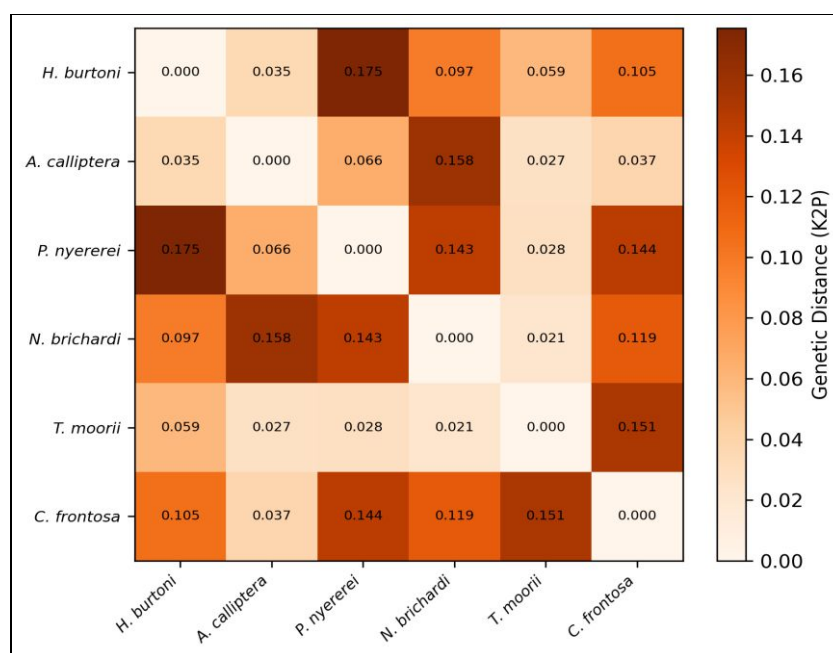


Figure 1. Pairwise genetic distance matrix (K2P model) for representative cichlid species across the three rift lakes. Tanganyika species showed the largest inter-specific distances, consistent with their older radiation.

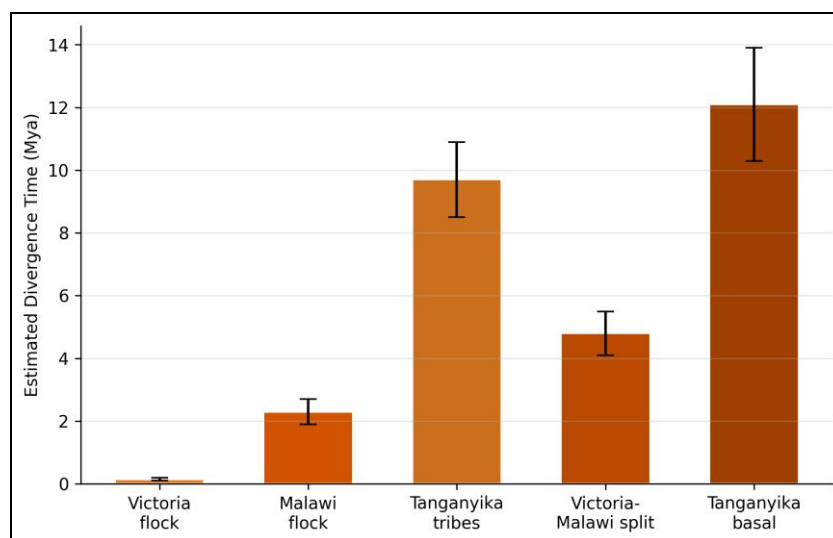


Figure 2. Estimated divergence times for major cichlid clades. Error bars represent 95% highest posterior density intervals. Note the logarithmic-scale difference between the Victoria superflock (0.15 Mya) and the Tanganyika basal split (12.1 Mya).

Discussion

The phylogenetic topology recovered here is broadly consistent with previous multi-gene analyses ^[2, 5]. The placement of Tanganyika tribes as basal supports the longstanding view that this oldest rift lake seeded the ancestor of the Victoria and Malawi radiations ^[1]. The Victoria superflock divergence time of 0.15 Mya falls within the range of estimates derived from geological evidence for the lake's last major desiccation event around 14,700 years ago ^[11], though our molecular estimate is somewhat older, possibly because mitochondrial divergence predates speciation in rapidly radiating lineages ^[3]. The Malawi flock age of 2.3 Mya is consistent with the lake's geological formation history and suggests a slower but still rapid diversification relative to typical vertebrate rates ^[12]. The dramatic compression of speciation events in Victoria—over 500 species in less than 200,000 years—argues that ecological opportunity in a newly formed lake, rather than time per se, is the principal driver of cichlid species proliferation ^[1, 4].

Conclusion

This multi-gene phylogenetic analysis confirmed the basal position of Tanganyika cichlid tribes and placed Victoria and Malawi flocks in a derived clade. Divergence time estimates ranged from 12.1 Mya for the oldest Tanganyika lineages to 0.15 Mya for the Victoria superflock, reinforcing the pattern that younger lakes harbor the most rapid radiations. The congruence between ML and Bayesian topologies lends confidence to the recovered relationships. These timelines support ecological opportunity as a primary driver of cichlid diversification and provide a temporal framework for comparative genomic investigations into the genetic basis of adaptive radiation.

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Contributions Not Qualifying for Authorship

Sequencing facility staff and colleagues who assisted with phylogenetic analyses are gratefully acknowledged.

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