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Ancient DNA is preserved in fish fossils from tropical lake sediments

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Abstract

Tropical freshwater lakes are well known for their high biodiversity, and particularly the East African Great Lakes are renowned for their adaptive radiation of cichlid fishes. While comparative phylogenetic analyses of extant species flocks have revealed patterns and processes of their diversification, little is known about evolutionary trajectories within lineages, the impacts of environmental drivers, or the scope and nature of now-extinct diversity. Time-structured palaeodata from geologically young fossil records, such as fossil counts and particularly ancient DNA (aDNA) data, would help fill this large knowledge gap. High ambient temperatures can be detrimental to the preservation of DNA, but refined methodology now allows data generation even from very poorly preserved samples. Here, we show for the first time that fish fossils from tropical lake sediments yield endogenous aDNA. Despite generally low endogenous content and high sample dropout, the application of high-throughput sequencing and, in some cases, sequence capture allowed taxonomic assignment and phylogenetic placement of 17% of analysed fish fossils to family or tribe level, including remains which are up to 2700 years old or weigh less than 1 mg. The relationship between aDNA degradation and the thermal age of samples is similar to that described for terrestrial samples from cold environments when adjusted for elevated temperature. Success rates and aDNA preservation differed between the investigated lakes Chala,

Kivu and Victoria, possibly caused by differences in bottom water oxygenation. Our study demonstrates that the sediment records of tropical lakes can preserve genetic information on rapidly diversifying fish taxa over time scales of millennia.

KEYWORDS
adaptive radiation, cichlid fish, conservation, diversification, evolution, palaeogenetics

1 | INTRODUCTION

The analysis of ancient DNA (aDNA) has revolutionized our understanding of evolution and has provided unique insights into past biodiversity and its geographic distribution through time, including species extinctions (e.g., Leonardi et al., 2016; Murray et al., 2017; Orlando & Cooper, 2014; Shapiro & Hofreiter, 2014; Thomas et al., 2019; Willerslev et al., 2014). While advances in aDNA extraction, sequencing and analysis have enabled genetic investigation of the remains of ancient humans and other large vertebrates from cold or temperate regions, this technology has yet to be applied to prominent study systems in speciation and evolutionary radiation research. In many of these study systems, such as Darwin's finches, *Heliconius* butterflies, Hawaiian silverswords or *Anolis* lizards, a challenge for palaeogenetics is the scarcity of well-preserved fossil remains. An exception to this general paucity of fossils from geologically young and extant radiations are the cichlid fishes of East Africa, which can leave chronologically highly resolved records of bones, bone fragments, teeth and scales in the sediments of lakes (Ngoepe et al., 2023; Cohen et al., 2016; Dieleman et al., 2015, 2019; Muschick et al., 2018; Reinthal et al., 2010). Yet, whether such remains contain appreciable amounts of endogenous aDNA is unknown. The high ambient temperature (~20°C–26°C) in the lower water column (hypolimnion) of these tropical lakes may be detrimental for aDNA preservation (Hofreiter et al., 2015), and other parameters of the depositional micro-environment, such as bottom-water pH or oxygenation, may affect the potential of long-term preservation (Allentoft et al., 2012; Kistler et al., 2017).

1.1 | The promise of tropical fish palaeogenetics

The Great Lakes of East Africa host globally unique communities of endemic fish species, which are of great scientific, economic and conservation interest. The adaptive radiations of cichlid fishes in these lakes have been investigated for their stunning arrays of morphologies, behaviours, nuptial colourations and ecological adaptations, their large species richness and, in some cases, their exceptional rate of evolutionary diversification (Muschick et al., 2012; Ronco et al., 2021; Salzburger, 2018; Seehausen, 2006). Highly resolved time series of genetic and phenotypic community-level data, alongside data on past environmental changes, are needed to better understand the dynamics of diversification in these adaptive radiations, including the role of extinctions and the effect of environmental changes (Cuenca-Cambronero et al., 2022). Some skeletal elements, such as certain teeth or cranial bones, can be identified to genus or even species level in some clades, but neither may this be possible in other clades, nor can it be achieved with the large majority of postcranial bones. While time series of phenotypic data on fossil remains can be informative about major ecosystem changes (Ngoepe et al., 2023), genetic data from these fish fossils may provide higher taxonomic resolution and allow many more aspects of long-term ecosystem dynamics and organismal evolution to be investigated. Depending on the study system and length of the time series, such data could reveal changes in community composition resulting from environmental perturbations or longer term evolution, both within individual lineages and across successive speciation events. Additionally, knowledge about the genetic diversity of ancient fish populations and their responses to past environmental changes can have important implications for the conservation and management of modern-day fish populations (Dietl & Flessa, 2011; Hofman et al., 2015; Nielsen & Hansen, 2008). Like lacustrine fish diversity elsewhere, the East African cichlid radiations are threatened by a range of factors. Introductions of non-native species, eutrophication due to nutrient pollution and global warming have all caused extinctions and the loss of genetic diversity (Kishe-Machumu et al., 2018; Seehausen et al., 1997). It is generally difficult to estimate the likely future impact of these factors on fish population sizes and species diversity. Therefore, palaeogenetic data from geologically young fossil records would help to appraise past functional and genetic diversity, establish baselines against which to compare current genetic diversity and possibly guide conservation measures (Atmore et al., 2022; Cohen, 2017; Dietl et al., 2015; Jensen et al., 2022; Kidwell, 2015).

1.2 | Exploring the feasibility of palaeogenetic analysis of tropical fish populations

Several studies have demonstrated the preservation of aDNA in fish remains, primarily from archaeological sites in temperate environments with samples that are hundreds to thousands of years old (Alonso et al., 2017; Atmore et al., 2022, 2023; Butler & Bowers, 1998; Ferrari et al., 2021; Kirch et al., 2021; Martínez-García et al., 2021; Oosting et al., 2019). Although poor preservation in tropical depositional contexts has been viewed as almost prohibitive to palaeogenetic

studies (Wade, 2015), Mergeay et al. (2006, 2007) successfully amplified nuclear microsatellites and mitochondrial DNA from up to 450-year-old resting eggs of *Daphnia* water fleas preserved in the sediment record of Lake Naivasha in Kenya. Further success with terrestrial and/or archaeological samples from tropical locations demonstrates that genome-level analysis is also feasible in some cases (Grealy et al., 2016; Gutiérrez-García et al., 2014; Kehlmaier et al., 2017; Woods et al., 2018). However, the burial environment in archaeological contexts is likely to be different from that in lake sediments, and aDNA preservation and successful retrieval in one context do not necessarily predict the same for the other. Fish remains from archaeological sites have revealed past trading routes (Star et al., 2017) and details about fishing practices or preferences in consumption (Häberle & Plogmann, 2019), but precisely because of these processes, those remains have become less informative about past community composition or population genetic structure. Additionally, archaeological deposits are often chronologically discontinuous, and the number of animal remains, including fish, available for destructive sampling can be limited (Alonso et al., 2017; Ferrari et al., 2021; Pálsdóttir et al., 2019). In contrast, fish remains from long, depositionally continuous lake records are often available in appreciable quantities (Ngoepe et al., 2023; Dieleman et al., 2015, 2019; Monchamp et al., 2021; Muschick et al., 2018). If the last few thousand years are of interest – to establish natural baselines of past taxon or genetic diversity prior to recent human impact, for example – samples can be accessed relatively simply through gravity or piston coring, and sample availability is mostly limited by the effort it takes to manually isolate them from the sediment matrix. Older samples, however, are often only accessible with major investment in deep drilling of ancient lakes (Cohen, 2012; Russell et al., 2020; Scholz et al., 2010; Verschuren et al., 2013). Inferring sample age from the position in the sediment column relies on age-depth models based on radiocarbon (^{14}C) or lead-210 (^{210}Pb) dates from the same sediment cores or cores from the same location in the lake. If such an age-depth model is not available and must be generated specifically for the purpose of dating the aDNA samples, this can substantially add to the costs and need for expertise. In principle, some effort could be saved by using bulk sediment as a source for aDNA, and such sedimentary ancient DNA (sedaDNA) has indeed been successfully extracted from tropical lake sediments (Boessenkool et al., 2013; Dommain et al., 2020; Epp et al., 2009, 2011). However, the downside to this approach is that it does not allow an individual-level assessment of past genetic diversity.

The degradation of endogenous DNA and the presence of large amounts of exogenous DNA make it difficult to assemble genomes de novo from typical aDNA sequence data. Hence, palaeogenomic approaches vitally depend on modern genomic reference data to identify and interpret endogenous sequence reads. As more and more whole-genome sequences of extant organisms are published, these can be leveraged for the analysis of aDNA. Joint efforts by the cichlid fish research community produced a small set of reference genomes that, given the close phylogenetic relationships within certain speciose adaptive radiations, provide an excellent resource for the study of most extant cichlid fish diversity (Brawand et al., 2014). Ongoing efforts by individual research groups have now re-sequenced additional hundreds of extant species from the East African Great Lakes Victoria (McGee et al., 2020), Malawi (Malinsky et al., 2018) and Tanganyika (Ronco et al., 2021), and such datasets will help to precisely map the genetic variation in cichlid aDNA data.

In this study, we evaluated whether fish remains from tropical African lake sediments yield endogenous aDNA. Following Kowalewski (2017), we use the term 'fossil' to include both fossils and subfossils, irrespective of their age and irrespective of the degree of diagenesis they have undergone. We extracted and sequenced DNA from fish bones, scales and teeth from the sediments of three East African lakes, with ages of deposition ranging from within the last decade (i.e., surficial bottom sediments) to ~20,000 years old. To increase the sensitivity of aDNA detection, we enriched a subset of sequencing libraries with target sequences using hybridization sequence capture. We then analysed read lengths and post-mortem damage and estimated the size of the endogenous fraction to evaluate the quantity and quality of aDNA in these samples. Finally, we attempted to taxonomically assign samples and place them in the phylogenetic context of modern relatives.

2 | MATERIALS AND METHODS

2.1 | Samples and sample preparation

Fish remains were collected from the sediments of three East African lakes: the two Great Lakes Victoria and Kivu, and Lake Chala, a crater lake at the foot of Mt. Kilimanjaro (Figure 1a). Core KIVU12-10A was accessed at the LacCore facility of the University of Minnesota (Minneapolis, USA). It had been collected by Robert E. Hecky and coworkers in Lake Kivu in 2012 from 210 m water depth in the Rwandan part of Lake Kivu (2°04′08.4″S, 29°18′42.1″E). We sampled this core for fish remains at 229.5–231 and 301–302 cm composite depths, and at 227–228.5, 232.5–233.5, 242.5–244.5 and 266–267 cm to obtain insect and terrestrial plant macrofossils for ^{14}C dating (Table S3). One additional fish fossil from Lake Kivu (Kivu 4) was collected from 38 to 40 cm depth in a gravity core of sub-recent sediment taken by Pascal Isumbisho Mwapu in 2006 in the Ishungu basin on the D. R. Congo side of the lake. Material from Lake Chala was extracted from three depth intervals of composite core CHA03/05, collected by the Challacea project from 94 m water depth near the centre of the lake (Verschuren et al., 2009). Material from Lake Victoria was extracted from two sets of cores: one set (LV95-1, LV95-2 and LV96-6) taken in Uganda in 1995 and 1996 by the IDEAL consortium and stored at LacCore, and the other (LVC18-1 and LVC18-2) taken in Tanzania in 2018 by a team from the University of Bern and EAWAG (Switzerland) and the Tanzania Fisheries Research Institute (TAFIRI). The fossils were extracted in the laboratory, either directly from the surface of split core sections using forceps or by screening wet-sieved fractions of bulk sediment retained on a 100 µm mesh. In total, 77 samples were analysed: four from Lake Kivu, 10 from Lake Chala and 63 from Lake Victoria; see Tables 1 and Table S1 for details.

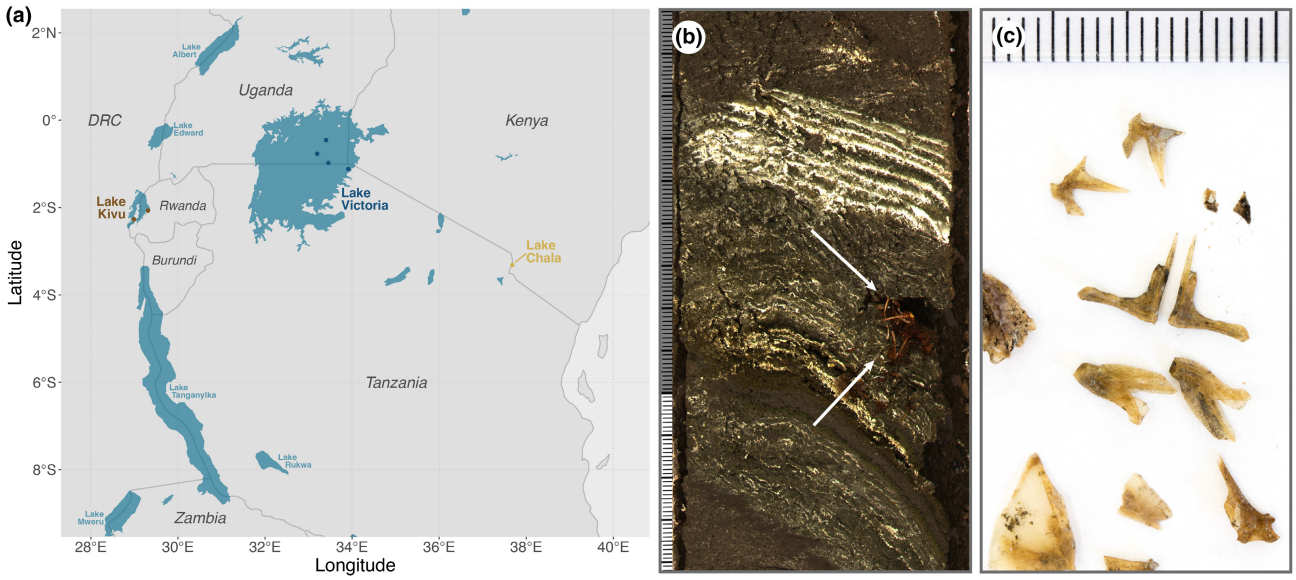

FIGURE 1 Distribution of lake sediment collection sites and examples of sedimentary fish remains (a) Map of equatorial eastern Africa with locations of analysed samples. For the large lakes Kivu and Victoria, the specific coring sites are indicated. Lake Chala is 2.5 km in diameter, too small to be visible at this scale. (b) Sediment core split surface of core KIVU12-10A section 3 showing a deposit of fish remains (white arrows, sample Kivu 1). Scale in mm. (c) Matched cranial fish bones of sample Kivu 1. Scale in mm.

TABLE 1 Age, weight and aspect data on taxonomically assigned fish fossils.

Sample	Type	Age [cal year BP] (95% CI)	Weight [mg]	Colour	Assigned taxon	*p*
Lake Chala						
Chala 1	Bones	770 (725–819)	*NA*	Light	Oreochromini	<.001
Chala 2	Bones	2628 (2588–2671)	*NA*	Light	Oreochromini	<.001
Lake Kivu						
Kivu 1	Bones	894 (787–1044)	100	Light	Haplochromini	<.001
Kivu 2	Bone	638 (579–685)	*NA*	Light amber	Haplochromini	<.001
Kivu 3	Bone	638 (579–685)	5	Light	Oreochromini	<.001
Kivu 4	Tooth	~240	*NA*	Light	Clariidae	.014
Lake Victoria						
Victoria 1	Bone	252 (−2–666)	1.5	Amber	Haplochromini	<.001
Victoria 2	Bone	15 (−68–182)	0.3	Amber	Haplochromini	<.001
Victoria 3	Bone	149 (−38–555)	2.3	Dark amber	Haplochromini	<.001
Victoria 4	Bone	149 (−38–555)	1.6	Dark amber	Haplochromini	<.001
Victoria 5	Bone	149 (−38–555)	1.2	Dark amber	Haplochromini	<.001
Victoria 6	Bone	149 (−38–555)	0.7	Dark amber	Haplochromini	<.001
Victoria 7	Scale	149 (−38–555)	0.3	Light amber	Haplochromini	<.001

Note: Sample ages are given in years before 1950 CE. The sample colour was assessed visually and qualitatively. Fossil weight indicated as NA refers to samples that had not been weighed before DNA extraction. Taxonomic assignment is based on a significantly higher number of reads mapping to a given reference as determined by both generalized linear models and adjustment of *p*-values for multiple comparisons (*p* values provided here) and a re-sampling test (results provided in Table S1).

Abbreviation: NA, not available.

2.2 | Age estimation of fish fossils

The age of fish fossils was inferred from age-depth models of their sedimentary context. No published age-depth model is available for core KIVU12-10A from Lake Kivu, and abundant evidence of depositional disturbance likely by turbidity flows (Robert E. Hecky, pers. comm.) precludes extrapolation of average sedimentation rates established for other deep-water coring sites in Lake Kivu to this location. We therefore ^{14}C-dated insect and terrestrial plant macrofossils from the core section bearing the extracted fish fossils

(Kivu 1–3) and constructed an age-depth model for that core section using first-order polynomial regression in Clam (Blaauw, 2010) with Intcal13 ^{14}C calibration (Reimer et al., 2013) on the measurements from six plant matter samples, omitting the insect sample as an outlier (Tables S4 and S5; Figure S2). We approximated the age of sample Kivu 4 by extrapolating a linear model of the ^{210}Pb-based age data on a gravity core collected nearby (also from the Ishungu basin; Pasche et al. (2010)). For Lake Victoria, we used published ^{14}C-based age models referenced in Berke et al. (2012) and Muschick et al. (2018) for the IDEAL cores from 1995 to 1996, or in Temoltzin-Loranca et al. (2023) for cores from 2018. The ages of fish fossils recovered from Lake Chala sediments are based on the age model of Blaauw et al. (2011).

2.3 | Ancient DNA extraction

Sediment samples were consistently handled in laboratory environments physically separated from facilities working with modern DNA. Extractions of aDNA and library preparations were performed in specialized aDNA facilities at the University of Oslo and ETH Zürich, which used high-efficiency particulate-absorbing (HEPA) filters, positive air pressure ventilation and overnight UV-C irradiation. Contamination with exogenous DNA was reduced by using standard aDNA laboratory procedures (Gilbert et al., 2006), such as wearing full body suits, face masks, gloves and frequent cleaning of surfaces and instruments using bleach and DNA Exitus Plus (Applichem). Cross-contamination between sediment samples was reduced by using single-use sterile forceps and containers. Before wet-sieving sediment samples, sieves were thoroughly rinsed and soaked in 2% sodium hypochlorite bleach. Wet-sieving was performed using demineralized water. In order to increase endogenous sequence yield per sequencing effort, we exposed subsets of samples to diluted bleach (0.5% NaOCl) or 10× diluted DNA Exitus Plus for 10–30 min, or to lysis buffer for 1 h (see Table S1 for details). Bleach and lysis buffer pre-treatment have been tested in previous studies (Boessenkool et al., 2016; Korlevic et al., 2015), but the use of diluted DNA Exitus Plus for the purpose of decontaminating sample surfaces is reported here for the first time to our knowledge. Possible advantages of DNA Exitus Plus over a treatment with diluted bleach are a milder effect on DNA (Fischer et al., 2016) and inactivation by ethylenediaminetetraacetic acid (EDTA), an ingredient in lysis buffer. We used established extraction protocols that first dissolve the sample in lysis buffer containing EDTA, Proteinase-K and N-Laurylsarcosine, and then reversibly bind DNA to a silica solid phase, provided either as silica powder dispersed in binding buffer (Brotherton et al., 2013) or as membrane in Minelute columns (Qiagen) (Gamba et al., 2015). For most samples, a molecular sieve (Amicon-4, 30 kDa, Millipore Inc.) was used to remove EDTA and salts from the lysate before binding to silica (Yang et al., 1998). DNA was eluted into TET buffer (10 mM Tris–HCl, 1 mM EDTA, 0.05% Tween-20 final concentration). We added two blank extraction controls to each batch of 12 samples. Details for each sample and control are provided in Table S1.

2.4 | Library construction, sequence capture, size selection and sequencing

We used both double-stranded DNA (dsDNA) library preparation after Meyer and Kircher (2010) and single-stranded DNA (ssDNA) library preparation following Gansauge et al. (2017, 2020) as described in Muschick et al. (2022). To sequence several pooled libraries on the same flow cell or lane, we labelled libraries with unique 7-bp indexed primers, either on the P7 side of fragments only (single-indexing) or on both the P7 and P5 sides (double-indexing; both indices unique for each library on a sequencing run) (Kircher et al., 2012; van der Valk et al., 2019). To each batch of 12 samples plus two extraction controls, we added one library negative control and one library positive control with an insert of a known sequence (CL104; Gansauge et al. (2017)). Details for each library are provided in Table S1. To increase the number of hits to the mitochondrial genome, we enriched a subset of libraries (Table S1) using one round of hybridization sequence capture following Gonzalez-Fortes and Paijmans (2019). Baits were created from the whole mitochondrial genome amplicons of *Pundamilia nyererei*, a member of the Lake Victoria region superflock of haplochromine cichlids. Libraries were captured either individually or as part of pooled reactions with 3–5 libraries per pool (Table S1). Prior to sequencing, we size-selected pools of amplified libraries to reduce both short-insert artefacts and long-insert modern contamination. Pools were size-selected by electrophoresis in 2% agarose gels, manual excision from the gel between the clearly visible adapter-artefact band and a 250-bp size marker, and subsequent DNA extraction from the gel slice using the QiaQuick Gel extraction kit (Qiagen). Alternatively, we used a BluePippin instrument (Sage Science Inc.) with a 3% gel cassette with internal markers, selecting for the size range 160–250 bp. We sequenced individual or pooled libraries on Illumina Miseq, NextSeq500 or Hiseq3000 platforms, either single-end or paired-end, with cycle numbers of 1 × 50, 1 × 75, 2 × 75 or 1 × 100 (see Table S1 for details). As is required for the single-stranded library builds with custom-shortened P7 adaptors, we used the custom sequencing primer CL72 (Gansauge & Meyer, 2013) and the custom index 2 read primer Gesaffelstein (Paijmans et al., 2017).

2.5 | Read filtering and mapping to reference genomes

Reads were demultiplexed using bcl2fastq v.2.19.1 (Illumina Inc.) with an allowed maximum combined distance of 1 when assigning barcode reads to samples. Even though sequencing was conducted for low cycle numbers only, the short insert sizes of aDNA libraries would lead to frequent reading into adapters. Therefore, we detected and removed partial adapter sequences from the reads using AdapterRemoval v2.0 (Schubert et al., 2016) and discarded reads shorter than 20 nucleotides in length after removal of adapter sequences. We then used the palaeomix pipeline (Schubert et al., 2014) to map reads to the individual reference genomes (Table S2),

choosing BWA v.0.7.17 (Li & Durbin, 2010) as the mapping engine with backtrack as the mapping algorithm (equivalent to setting 'bwa-aln'), and a Phred mapping quality threshold of 25. First, we mapped a selection of mitochondrial reference genomes to taxonomically assign samples. Mitochondrial references included one species from each of the 15 extant, native fish families inhabiting one or more of the study lakes, with Cichlidae being represented by two tribes, Haplochromini and Oreochromini (Table S2). Mitochondrial reference genomes varied in length, with most being close to the average of 16,600 bp, except for *Protopterus aethiopicus* having a shortened and *Nothobranchius furzeri* having an extended control region. In order to directly compare the number of unique hits across references, we shortened sequences to the first 15,000 bp for taxonomic assignment (see Section 2.6), omitting the control region while retaining protein-coding genes. For phylogenetic analyses, reads were mapped to full-length sequences. Taxonomically assigned samples were further mapped to the nuclear genomes of a representative species from the assigned taxon to estimate the fraction of endogenous DNA present and to assess post-mortem damage patterns. To decrease spurious read mappings, low-complexity stretches in the nuclear reference genomes were hard-masked using Dustmasker (Morgulis et al., 2006) at level 15.

2.6 | Taxonomic assignment

Samples were assigned to the taxon that produced the highest number of hits if the difference in hit counts compared to other references was statistically significant. To assess the significance of read count differences, we used two methods: (1) generalized linear models with Poisson error distribution and log link function followed by adjustment of *p*-values for multiple comparisons using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995); and (2) a resampling test, where for samples with more than 100 total hits, the hits across references were joined and re-sampled with replacement 1000 times. In the resampling test, the number of times a taxon is returned as most abundant across the 1000 resamplings is then used to calculate the *p*-value for the assignment. The use of two methods allowed to gauge the robustness of the assignment. So-called index-hopping (van der Valk et al., 2019) can lead to sequences from one sample being associated with another sample or control on the same sequencing run. This can be problematic, especially in aDNA studies, if samples vary widely in their read numbers and endogenous content. We therefore removed hits that were shared between libraries within sequencing runs before taxonomically assigning samples and disregarded libraries that shared ≥10% of their hits with other libraries, as this indicates that they had likely received a large fraction of their total hits from other libraries. Only samples for which taxon assignment was successful were analysed further; all other samples were designated as 'empty'. Statistical evaluation of read counts and adjustment for shared reads was performed in R v4.2.2 (R Core Team, 2022) using the package Rsamtools v2.14 (Morgan et al., 2022) and custom scripts.

2.7 | Read length and post-mortem damage pattern assessment

To investigate the relative preservation of aDNA in different lakes, we determined the distributions of lengths of unique hits against reference genomes for taxonomically assigned samples. Counts of unique hits by read length were smoothed by fitting a Gaussian loess curve using the 'loess' function with 'span' parameter set to 0.5 (R Core Team, 2022). The correlation of the medians of these distributions with sample age was then investigated with second-order polynomial regression. We assessed post-mortem damage patterns stemming from cytosine deamination by calculating the relative prevalence of apparent C to T and G to A changes from reference to sample by nucleotide position in reads, both from the 3′-end and the 5′-end, using mapDamage v2.0.8 (Jónsson et al., 2013). We also estimated both the distribution of single-stranded molecule ends (i.e., overhangs) and the proportion of deaminated cytosines within them (δ_s) using mapDamage following Kistler et al. (2017), and compared our estimates to a model of Kistler et al. (2017). That model is based on 185 palaeogenomic datasets from mammal bones and describes a relationship between δ_s and sample age for a given mean ambient temperature. We adapted this model for 24°C, the approximate average temperature at the water depths of coring sites in lakes Victoria (Hecky et al., 1994) and Kivu (Katsev et al., 2014); in Lake Chala, the deep-water bottom temperature is slightly cooler, about 22°C (Buckles et al., 2014; van Bree et al., 2020).

2.8 | Phylogenetic analyses

We placed fossil samples containing endogenous aDNA in the context of extant species to reveal their phylogenetic association and potentially their species identity. Taxonomically assigned samples were mapped to a representative mitochondrial genome of their taxon: *Oreochromis tanganicae* for Oreochromini, *Pundamilia nyererei* for Haplochromini and *Clarias gariepinus* for Clariidae (see Table S2 for details). We used an iterative mapping strategy (as has been used elsewhere, e.g., Hahn et al., 2013; Westbury & Lorenzen, 2022) with the following steps: adapter sequences were eliminated from sequencing reads, and paired-end reads were merged using AdapterRemoval v2.0 (Schubert et al., 2016). Output from an initial mapping using BWA (settings aln -t 2 -n 0.05, seeding disabled) was deduplicated using Samtools (Li et al., 2009), then called with ANGSD v.0.933 (Korneliussen et al., 2014) with a minimum depth of 3. Those calls were pasted into the initial reference, and this chimeric sequence was used as a new reference. The iterative part of the mapping consisted of two-part iterations and also used BWA, but with relaxed settings (–n 0.01 -o 3). The first part of each iteration called using GATK's (v.4.1.2.0, McKenna et al., 2010) HaplotypeCaller, then produced a new reference using GATK's FastaAlternateReferenceMaker, while the second part used again ANGSD for calling and merged the calls with the previous reference using R. The process continued until no additional reads

could be mapped. The final bam file was then called using ANGSD, but without merging with a reference, therefore retaining only calls supported by the sample's sequence reads. The extant species chosen to provide phylogenetic context are representative of the diversity within the clades of interest, i.e., to which fossil samples have been taxonomically assigned, and inhabit the respective study lakes or nearby water bodies. For Lake Chala, we included the native and endemic *Oreochromis hunteri*, its putative sister species *O. jipe*, which occurs in the nearby Pangani River drainage, and *O.* sp. 'bandia', which was recently introduced into Lake Chala and has also been called *O.* sp. 'blue head' (Moser et al., 2018) or *O.* cf. *korogwe* (Dieleman et al., 2018). The three species were represented by sequences of two mitochondrial loci (1486 bp combined, from Dieleman et al., 2018), not the whole mitochondrial genome. For Lake Kivu, the native *O. niloticus* was represented with several specimens from different localities throughout its range of distribution; however, none were available from Lake Kivu itself; and *Haplochromis paucidens* and *H. vittatus* represented the native haplochromine radiation that is part of the Lake Victoria region superflock. Lake Victoria's haplochromine adaptive radiation was represented by one species each from seven genera. Whole mitochondrial genomes were available on GenBank (www.ncbi.nlm.nih.gov/genbank/) or were reconstructed using the iterative mapping approach described above using available short reads (details are given in Table S2). Sequences of modern representatives and fossil samples with more than 500 called sites were aligned in mafft v.7.273 (Katoh & Standley, 2013) using default settings. Phylogenies were calculated using RAxML v.8.2.4 (Stamatakis, 2014) with GTRGAMMA as a substitution model.

3 | RESULTS

3.1 | Sequencing results and taxonomic assignment

Out of 77 analysed fish fossils, 13 yielded endogenous aDNA sequences and could be assigned to family or tribe level (Table 1). The two methods used for testing the significance of taxon assignments based on hit counts were in agreement, with only minor differences in the probability of assignment (Table S1). Of these 13 taxonomically assigned fish fossils, at least 11 represented individuals with distinct mitochondrial haplotypes. The other two, Victoria 6 and 7, had only a few sites called, none of which defined an additional haplotype, but leaving open the possibility that their sequences differed at non-called sites. These 13 successful assignments include samples from each of the three study lakes, and their ages range from a few decades to ~2700 years. Three different taxa were identified: oreochromine and haplochromine cichlids, and a clariid catfish (Table 1). Mapping against reference genomes of extant taxa inhabiting the respective lakes or their close relatives revealed an endogenous content of up to 11.8% (Table 2), while 64 samples produced either too few hits or, inconclusively, a similar number of hits to several phylogenetically distant reference genomes and thus could not be assigned unequivocally (Table S1). The ratio of assigned to unassigned samples differed between lakes Chala (2/10, or 20%), Kivu (4/4, or 100%) and Victoria (7/63, or 11%) (Figure 2). Sequence capture increased the fraction of non-deduplicated reads mapping to the respective mitochondrial genome up to 8143-fold (Table 2 and Table S1). The enrichment of four samples (Victoria 1 to 4) taxonomically assigned to Haplochromini and sequenced both with and without sequence capture ranged from 999-fold to 8143-fold, median 1076-fold. Kivu 3, assigned to Oreochromini, was enriched 233-fold by sequence capture using baits created from a haplochromine mitochondrial genome. The endogenous content of assigned samples ranged between 0.01% and 12.6% for sequence-captured libraries and between 0.05% and 11.8% without capture. Samples from Lake Victoria had markedly lower endogenous content on average than samples from the other two lakes (median, no capture: Lake Victoria 0.05%, Lakes Kivu and Chala 7.6%). The four different sample pre-treatments prior to extraction (i.e., no pre-treatment, incubation in lysis buffer, diluted bleach or diluted DNA Exitus Plus) all produced positive samples. Lake Victoria samples treated with DNA Exitus Plus yielded more positive samples (6 out of 12) than bleach and lysis buffer pre-treatments combined (1 out of 51), but this is highly conflated with sample age (Table S1).

3.2 | Age of fish fossils

The absolute mean age of the 63 samples from Lake Victoria analysed in this study ranges from 15 to 19,540 cal year BP (see Tables 1 and Table S1 for 95% confidence intervals, CI). However, the samples' ages are bimodally distributed, with 46 being older than 10,000 years old and 17 younger than 1000 years (Figure 2). New ^{14}C dates on core KIVU12-10A place the mean ages of samples Kivu 1–3 between 638 and 894 cal year BP, with a 95% CI ranging from 579 to 1044 cal year BP. The age of sample Kivu 4 is estimated as ~240 cal year BP. The Lake Chala samples have mean ages ranging from 770 to 7924 cal year BP (see Tables 1 and Table S1 for 95% CIs). The age of the 13 fish fossils that contained endogenous DNA and could be taxonomically assigned was on average the youngest in Lake Victoria (mean ages up to 252 cal year BP, or ~ 320 years old), intermediate in Lake Kivu (up to 894 cal year BP, or ~ 960 years old) and oldest in Lake Chala (up to 2628 cal year BP, or ~2700 years old; Figure 2).

3.3 | Post-mortem damage

When summarizing differences between mapped reads and references by position within reads from either end, the typical post-mortem damage pattern caused by the deamination of cytosine emerges. Apparent changes of C to T and G to A are particularly prevalent in the ends of aDNA molecules (Figure 3a) and can indicate their authenticity. All taxonomically assigned fossil samples show this pattern, although to varying degrees. Taxonomically assigned samples from lakes Chala and Kivu are older and, accordingly, show more pronounced deamination than those from Lake Victoria. Comparing deamination in

TABLE 2 Summary statistics of aDNA sequencing and mapping results.

Sample	Library	Seq. Run	Sequence capture	Assigned taxon	Retained reads	Avg. hit length	Fraction shared hits	Endogenous fraction [%]	Mito. Genome coverage	Sites called	Weight [mg]
Lake Chala											
Chala 1		2	No	Oreochromini	7,349,755	58.2	0.055	11.75	0.63	2243	*NA*
Chala 2		2	No	Oreochromini	7,766,237	47.9	0.064	10.73	0.28	780	*NA*
Lake Kivu											
Kivu 1		1	No	Haplochromini	15,088,024	41.0	0	1.23	4.73	13,308	100
Kivu 2		2	No	Haplochromini	56,005,290	48.7	0.038	0.19	1.76	5898	*NA*
Kivu 3	1	6	Yes	Oreochromini	6,436,533	48.8	0.001	12.59	34.96	16,565	5
Kivu 3	2	3	No	Oreochromini	383,506		0	5.34	0.22		
Kivu 4		7	No	Clariidae	967,253	66.4	NA	9.96	0.12	5	*NA*
Lake Victoria											
Victoria 1		5	Yes	Haplochromini	12,126,691	60.2	0.063	0.24	22.49	16,747	1.5
Victoria 1		4	No	Haplochromini	947,560		0	0.05	0.14		
Victoria 1		3	No	Haplochromini	172,658		0	0.05	0.02		
Victoria 2		5	Yes	Haplochromini	399,218	64.8	0.006	0.29	6.9	16,083	0.3
Victoria 2		6	No	Haplochromini	3,769,375		0	0.05	0.04		
Victoria 3		5	Yes	Haplochromini	1,297,369	64.1	0.01	0.79	4.35	12,838	2.3
Victoria 3		6	No	Haplochromini	4,916,489		0	0.18	0.04		
Victoria 4		5	Yes	Haplochromini	1,455,924	63.1	0.024	1.15	2.91	8822	1.6
Victoria 4		6	No	Haplochromini	4,772,194		0	0.20	0.02		
Victoria 5		5	Yes	Haplochromini	390,329	57.3	0.004	0.07	0.92	1304	1.2
Victoria 6		5	Yes	Haplochromini	558,692	59.6	0.026	0.01	0.49	397	0.7
Victoria 7		5	Yes	Haplochromini	414,006	52.6	0.024	0.02	0.31	90	0.3

Note: One library was built per sample, except for Kivu 3, for which two libraries were built. Several samples were sequenced multiple times, often once with and once without target enrichment by sequence capture. The fraction of shared hits denotes the fraction of hits with identical sequences shared with other libraries on the same sequencing run, likely a consequence of index-switching. The endogenous fraction is the percentage of reads mapping to the respective nuclear reference genome before filtering duplicates.

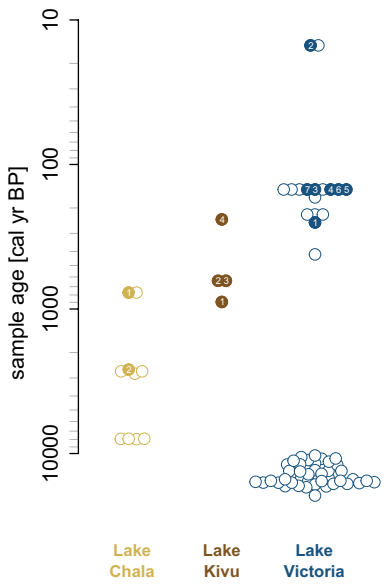

FIGURE 2 Success of taxonomic assignment against fish fossil age. Filled circles represent taxonomically assigned samples, and empty circles represent non-assigned samples. Note the logarithmic age scale; cal year BP, calibrated years before 1950; noise was added to age estimates of samples older than 10,000 cal year BP for illustrative purposes.

single-stranded overhangs of fragments (δ_s) to a model based on a large amount of mammal-bone data from terrestrial sampling sites shows that the amount of damage in our samples falls largely within the wide 95% CI of the prediction (Figure 3b) but tends to be elevated within that range, especially those from Lake Victoria. Read lengths were short, as expected for aDNA, with a mean length between 47.9 and 79.5 bp for the 13 assigned samples (Figure 3c). Interestingly, while modes of read lengths ranged between 25 and 69 bp for all assigned samples, and most samples' read counts decrease steeply with increasing read length, Kivu 4 shows an extended tail in its read length distributions, reaching appreciably beyond 100 bp. Samples from Lake Victoria show no clear peak in their distributions, but rather an extended plateau. Median fragment lengths are negatively correlated with sample age ($F_{(2,10)}=6.78$, $p=.02$, adj. R-squared$=.49$), with the fragment size declining steeply with age in samples younger than 1000 years and apparently remaining constant beyond that age (Figure S1).

3.4 | Phylogenetic analyses

Among the 13 taxonomically assigned fish fossils, 10 yielded sufficient data to place them in the phylogenetic context of extant species (Figure 4). Victoria 6 and 7 and Kivu 4 had respectively 90, 397 and 5 mitochondrial genome positions called and were excluded from phylogenetic analysis. Kivu 1 and 2 were identified as members of the tribe Haplochromini, which is represented by 15 described and some closely related undescribed species in Lake Kivu today (Snoeks et al., 2012). Both samples cluster with the two extant Lake Kivu representatives of the Lake Victoria Region haplochromine superflock, which are closely related to superflock species from Lake Victoria itself. Kivu 3 was assigned to the cichlid tribe Oreochromini. It robustly associates with *Oreochromis niloticus* (bootstrap support value: 97; see Figure 4), which is thought to be native to Lake Kivu, while the three other Lake Kivu *Oreochromis* species used here for context (*O. mweruensis*, *O. macrochir* and *O. leucostictus*) are introduced. The situation is similar for the two assigned fossils from Lake Chala, which cluster with both *O. hunteri*, the only Lake Chala endemic, and *O. jipe*, the sister species to *O. hunteri* inhabiting the nearby Pangani River drainage. Modern reference data for *O. hunteri* and *O. jipe* was available from two mitochondrial loci only (1486 bp combined (Dieleman et al., 2018)); hence, there is limited sequence overlap between the fossils and modern specimens. Given the shallow divergence between these two extant species, fossils could therefore not be assigned decisively to either species. Another *Oreochromis* species found in Lake Chala, *O.* sp. 'bandia', is likely a recent introduction (Dadzie et al., 1988). Fossil samples from Lake Chala do not cluster with either of the two divergent haplotype groups in that population. The five samples from Lake Victoria suitable for phylogenetic analysis clustered with representatives of the Lake Victoria radiation of haplochromines. Thus, despite the shallow divergence between haplochromine cichlids from Lake Kivu and Lake Victoria (Meier et al., 2017), fossils clustered with the fauna of their respective lakes. Within Lake Victoria, haplochromine species are only weakly genealogically sorted in their mitochondrial haplotypes, a result of both the young age of this radiation and more recent interbreeding caused by habitat deterioration (Seehausen et al., 1997), preventing an assignment of haplochromine fossils to genus or species level based on mitochondrial data alone.

4 | DISCUSSION

4.1 | Taxonomic assignment and phylogenetic placement of fish fossils

Overall, 17% ($n=13$) of the analysed fish fossils were found to contain endogenous DNA and could be taxonomically assigned to the tribe or family level. Of these, we included those 10 samples that had at least 500 mitochondrial genome positions called in our phylogenetic analysis (Figure 4), where they clustered conclusively with species native to their respective lakes (Figure 4), even in the case of the haplochromines from Lake Kivu and Lake Victoria, which are closely related to one another (Meier et al., 2017). The association of fossils from Lake Chala with *Oreochromis jipe* and *O. hunteri* confirms previous assessments based on fishery surveys (Dadzie et al., 1988; Lowe, 1955; Trewavas, 1966, 1983) reports by local fishermen and morphological analysis of modern and fossil teeth (Dieleman et al., 2015) that only *O. hunteri* is native to Lake Chala and *O.* sp. 'bandia' introduced. These phylogenetic associations are not surprising and do not yield new insights into fish phylogeography, but they do serve as evidence that the genetic data we retrieved from the fossils is endogenous and not the result of exogenous contamination or cross-contamination between samples.

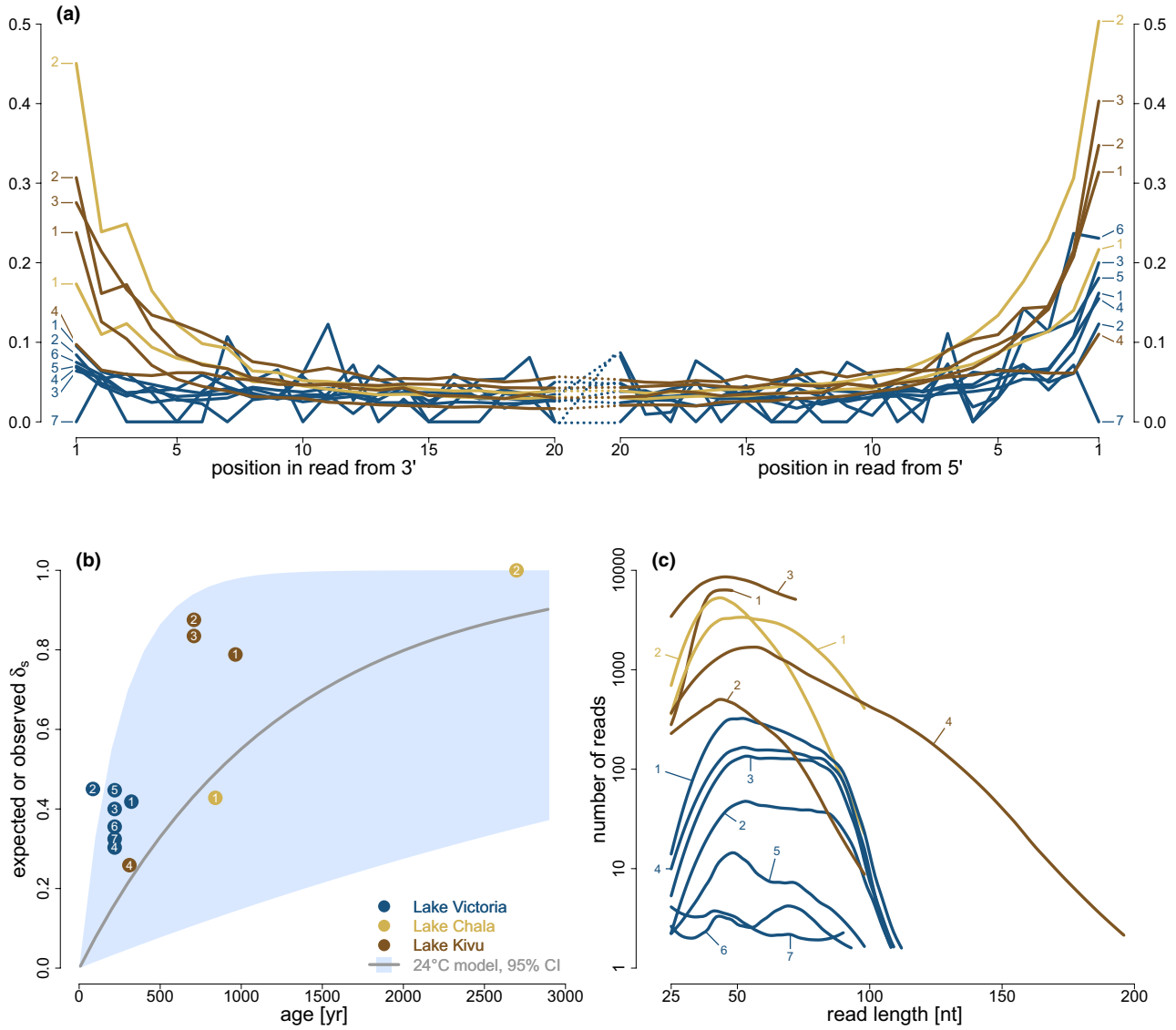

FIGURE 3 Post-mortem damage in mapped reads of taxonomically assigned fish fossils. (a) Post-mortem damage from cytosine deamination is distributed unevenly along mapped sequencing reads. The combined fraction of T in reads where the reference is C, and A where the reference is G, is plotted against the position in mapped reads either counted from the 3′-end or the 5′-end. As this chemical alteration is especially prevalent in single-stranded overhangs, the relative abundance of apparent C > T and G > A changes at reads' ends is indicative of authentic ancient DNA. Dotted lines connecting the left and right parts of each plot are for illustration purposes only. (b) Proportion of deaminated cytosines in single-stranded overhangs (δ_s) in individual samples, and model of expected δ_s by sample age for 24°C ambient temperature in a terrestrial context. (c) Read length distributions of mappings of taxonomically assigned samples against their respective nuclear reference genomes. Artefactual peaks from inserts exceeding the maximum read length were omitted by disregarding counts in the last 3 bp bins. Read lengths are short, as is typical for aDNA. The legend in panel b applies to all panels. CI, confidence interval; nt, nucleotides.

4.2 | Taphonomic considerations

Lake sediments often contain an abundance of fish remains (Ngoepe et al., 2023; Dieleman et al., 2015; Muschick et al., 2018), which have most often been buried as dispersed individual elements, namely scales or teeth shed over an individual's lifetime, or parts of decomposing carcasses following their fragmentation by bottom currents and bioturbation. Occasionally, whole carcasses can be embedded in situ, with the skeletal parts staying articulated or in close contact. Our collection of analysed fish fossils contained both types. The different bones comprising sample Kivu 1 most likely belonged to the same individual (Figure 1c), as indicated by laterally matching cranial elements. We also did not find any variable sites in this sample's mitochondrial genome sequence that could not be explained by post-mortem damage. If found, such sequence variation would indicate the presence of different haplotypes among the bones, which would most likely stem from multiple individuals. Other remains found together within a single sediment core interval did clearly belong to different individuals: samples Kivu 2 (a nondescript rib bone) and Kivu 3 (a left

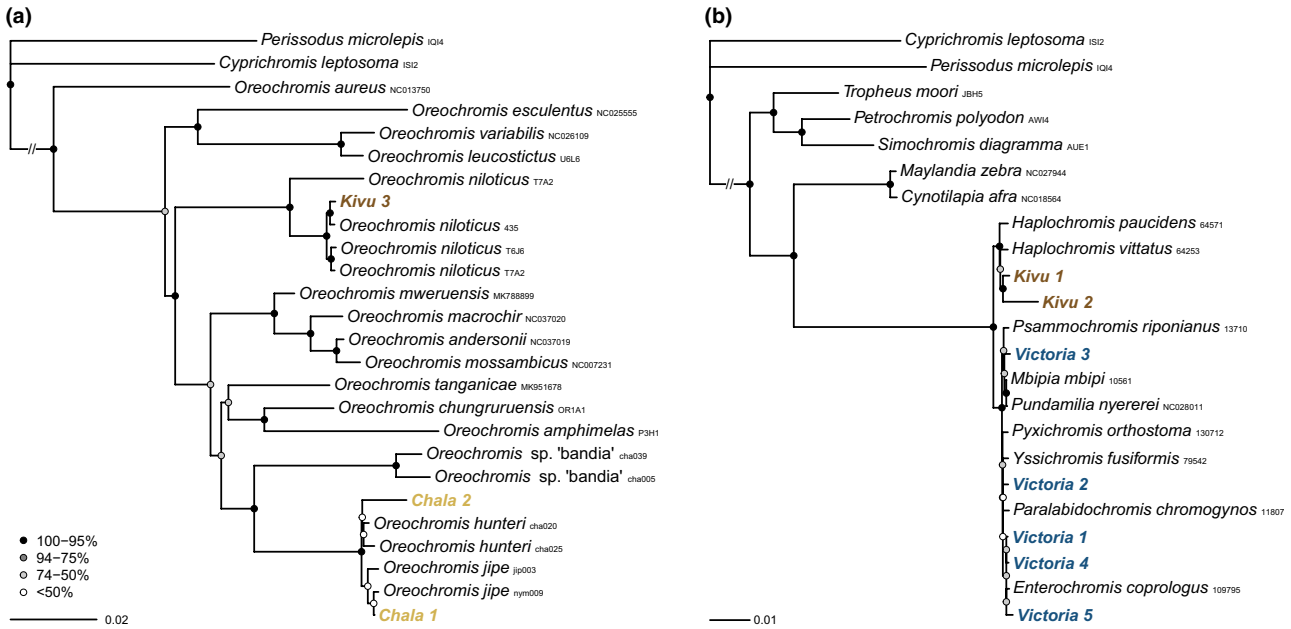

FIGURE 4 Phylogenies of ancient fish fossils in the context of modern fish diversity in the three East African study lakes. (a) Oreochromine fossils from lakes Kivu and Chala in the context of East African *Oreochromis* species. (b) Haplochromine fossils from lakes Tanganyika, Malawi, Victoria and Kivu. Phylogenies are based on whole mitochondrial genome sequences for modern specimens, except for *O.* sp. 'bandia', *O. hunteri* and *O. jipe*, for which ND2 and D-loop sequences were used. The number of called sites for fossils is given in Table 2. Nodes are labelled according to their support from 100 bootstrap replicates. Scale bars indicate estimated substitutions per site.

premaxilla) were identified as Haplochromini and Oreochromini, respectively (Table 1). Samples Victoria 3–7 were all found at the same sediment depth (22.2 cm) in the same core (Table S1), and yet Victoria 3, 4 and 5 have different mitochondrial haplotypes (Figure 4). Samples Victoria 6 and 7 had too low mitochondrial genome coverage to make this assessment. For the analyses of fish fossils from sediment cores, this result is enlightening, as it suggests that fish remains buried within a short time interval across a sediment surface area of 28–31 cm^2 (60–63 mm core diameter) may include many individuals and, at least in Lake Victoria, are not derived from a single carcass. In many cases, it is impossible to confidently determine by their morphology whether teeth, scales, bones or bone fragments found together belonged to one individual or several, especially in the case of very closely related cichlid fishes of similar size. Our findings suggest that raw counts of disarticulated fish fossils may be used as an approximation of the number of contributing individuals, whereas laterally matching cranial bones or an abundance of similarly sized vertebrae indicate the local burial of an intact carcass.

4.3 | Degradation state of endogenous aDNA and potential determinants of its preservation

4.3.1 | Differences in DNA degradation

Sequence reads mapping to reference genomes were short and showed a distinct pattern of post-mortem cytosine deamination (Figure 3), both suggesting that the DNA we recovered from the analysed fish fossils is authentically ancient. Moreover, the length of mapped reads from the taxonomically assigned samples is inversely correlated with sample age (Figure S1). This result is expected, given that DNA breaks down into smaller fragments after the organism's death. However, other studies found no correlation of this sort and interpreted this as the result of initially rapid, then slowing fragmentation (Kistler et al., 2017; Pääbo, 1989; Sawyer et al., 2012). Our results indicate that DNA degradation in tropical lake sediments may slow down after about 1000 years, albeit this inference hinges on a single data point older than this possible threshold age (Figure S1). In any case, it is possible that the age range of our samples encompasses the early period of rapid DNA fragmentation. In terrestrial contexts, the degree of cytosine deamination in single-stranded overhangs (δ_s) appears strongly related to age and ambient mean temperature, jointly considered as the thermal age (Kistler et al., 2017). This model is generally supported by our data. However, when accounting for sample age, the δ_s values of our Chala samples are close to the model mean, whereas most Victoria and Kivu samples have more elevated δ_s values situated near the fringes of the model's 95% CI (Figure 3b).

Further, taxonomically assigned samples from Lake Victoria have a lower endogenous DNA content than samples from the other two lakes (Table 2), to the degree that the Victoria sample with the highest endogenous content (Victoria 4: 0.2% for measurement without sequence capture) is on par with the lowest among the Kivu and Chala samples (Kivu 2: 0.19%). This is in line with the ratios of assigned to unassigned (i.e., dropped-out) samples across lakes, which

are lower for Victoria (0.11) than for Chala (0.2) and Kivu (1.0). All four measures – sample dropout, endogenous content, deamination and fragmentation – are indicators of the severity of DNA degradation. Except for fragmentation, these suggest that the DNA in the Lake Victoria samples is more strongly degraded than that from the other two lakes. This could be due to a number of reasons, including both genuine sample- or environmental-related factors and biases due to variation in experimental procedures, as discussed below.

4.3.2 | Experimental factors

Size-selective steps in DNA extraction, library preparation or sequence capture may have affected the length distributions of mapped sequences in different ways. Thus, one explanation for the plateau-shaped read length distribution of the seven taxonomically assigned samples from Lake Victoria (Figure 3c) could be an effect of hybridization sequence capture, which proved particularly effective for these samples. In that case, a bias in sequence capture to retain longer fragments would shift the mean and median mapped read length of these (relatively young) samples compared to those of less efficiently captured samples and those that did not undergo sequence capture. Another possibility is that because taxonomically assigned Victoria samples had been isolated from the sediment soon after core collection (all originate from the 2018 cores; Table S1), less DNA fragmentation might have occurred ex situ than in other samples. We consider the latter scenario less likely, as it is contradicted by the other measures of DNA degradation. In contrast to Victoria samples, the mapped read lengths of taxonomically assigned samples from lakes Chala and Kivu show more narrow unimodal distributions (Figure 3c). In most cases, the maximum read length appears to be related to the number of cycles in sequencing, with longer fragments in Kivu 4 being revealed as such by the greater number of cycles used for this sample (Table S1). However, fragments exceeding ~150 bp are rare even in this young and comparatively well-preserved sample. Storage times and conditions after excavation can be important factors for DNA preservation in fossils (Pruvost et al., 2007). While most fish fossils for this study were extracted from the sediment multiple years after core collection (up to 22 years in the case of Lake Victoria cores from the IDEAL project), fish fossils from Lake Victoria cores collected in 2018 had been ex situ for the least amount of time (1 year) and were continuously stored under refrigerated conditions, yet they exhibit worse preservation than samples from the other lakes. Thus, it appears that differences in storage conditions do not explain much of the variation in DNA preservation among our samples.

Prior to DNA extraction, we pre-treated subsets of samples alternatively with bleach (47 samples), lysis buffer (13 samples) or a diluted DNA Exitus Plus solution (12 samples) (Table S1), with the intention of increasing the fraction of endogenous DNA by removing exogenous DNA from the sample surface. However, when DNA is destroyed indiscriminately, it can also cause a low yield overall (Basler et al., 2017; Boessenkool et al., 2016; Korlevic et al., 2015). The available data presented here precludes an assessment of which treatments proved most effective and whether they may have led to a low yield or increased sample dropout because the sample treatment scheme did not specifically aim for a comparative evaluation of the different treatments. As all treatments produced positive samples, we suspect that the differences between them may not be large. Further tests are needed to establish the relative effectiveness of these treatments for small fish remains from tropical lake sediments.

4.3.3 | Environmental and sample-specific factors

Humic and fulvic acids, and/or mineral compounds such as iron or manganese oxides, are known to invade fossil bones buried in soils (Kendall et al., 2018) and sediments (Stathopoulou et al., 2013), and, when co-extracted with the aDNA, can decrease the efficiency of library preparation. The type and degree of this alteration are visible as a staining of the bone material from light shades of brown or amber to progressively deeper shades of brown to black. The fish fossils analysed in this study appear to differ between the lakes in the degree of staining, with most Victoria fossils being a shade of amber or dark brown, while most Kivu and Chala fossils are less stained (Table S1). Differences in the degree of inhibition of enzymatic steps during library preparation may explain variation in the ratio of assigned to unassigned samples across lakes but would not readily account for the large range in the endogenous fraction of positive samples (Table 2: 0.01%–12.6%).

The average mass of fossils for which the weight was measured was lower for samples from Lake Victoria than for lakes Kivu and Chala (1.8 mg; versus 4.6 and 52.5 mg). In the case of northern European herring bones from archaeological settings, weighing from <10 to 70 mg, Atmore et al. (2023) found no clear correlation between aDNA preservation and fossil weight except for a drop in the fraction of samples yielding endogenous DNA for samples weighing <10 mg. As the weight range of fossils in our study is fairly large (0.3–100 mg), sample weight may conceivably have been a factor in aDNA preservation in the smallest samples; however, the volume of our data does not allow us to evaluate this effect statistically.

Water temperature differs between lakes, with Kivu and Victoria averaging about 24°C at the depths of the respective coring sites (Hecky et al., 1994; Katsev et al., 2014), whereas the bottom of Lake Chala is ~2°C cooler (Wolff et al., 2014). Temperature is an important determinant of DNA degradation (Bollongino et al., 2008; Dabney et al., 2013), and a positive relationship has been shown with the rate of cytosine deamination (Kistler et al., 2017). However, a difference of 2°C is unlikely to account for the poorer aDNA preservation in samples from Lake Victoria.

Oxygenation of the sediment surface at the location of sample deposition and burial does differ between the three study lakes in a way that may explain differences in DNA preservation. Lake Chala is permanently stratified (meromictic) with its water column anoxic below 15 and 45 m depth depending on the season (Wolff

et al., 2014), and the Challacea coring site from which analysed fossils were recovered is located at 94 m depth. Lake Kivu is also meromictic and anoxic below 30–65 m water depth depending on the season (Pasche et al., 2012), with coring sites located at ~170 m (Kivu 4) and 210 m depth (Kivu 1–3). In contrast, with a maximum water depth of 68 m, Lake Victoria is relatively shallow for its large surface area, and prior to the development of hypolimnetic anoxia associated with cultural eutrophication, its lower water column used to be oxygenated year-round (Hecky et al., 1994). Once they are covered by a few centimetres of sediment, fossils are shielded from oxygen when bottom waters are oxygenated. But depending on the sedimentation rate, the time until a fossil is buried sufficiently deeply can be substantial. Bioturbation can extend this period further as it transports oxygen into the sediment. It may then take many decades until samples experience anoxic conditions. The presence of oxygen may be detrimental to aDNA preservation in fish fossils in two ways. Firstly, organic matter decomposition in an oxygenated environment typically lowers the pH, directly increasing DNA degradation by hydrolysis (Bollongino et al., 2008; Mitchell et al., 2005). Secondly, both mineral and protein components of fish remains preserved better when embedded under suboxic, reducing conditions, as was shown for marine environments (Aguilera et al., 2016; Díaz-Ochoa & Pantoja, 2014; Schenau & Lange, 2000). Protection of the DNA against chemical or microbial decay provided by the matrix of bones or teeth is then maintained for longer (Campos et al., 2012).

4.4 | Conclusions and outlook

The results of this study represent the first demonstration of ancient DNA preservation in sediment-embedded fish remains from tropical lakes. To the extent possible, we also assessed potential causes of variation in the samples' endogenous DNA content and its state of degradation. Although the results of this study may have been affected by differences in sample pre-treatment and processing, the overall picture that emerges is that fish fossils from lakes Chala and Kivu preserved ancient fish DNA better than those from Lake Victoria. The long-term oxygenation of Lake Victoria bottom waters, contrasting with permanently anoxic conditions in Chala and Kivu, may provide the most plausible explanation for this difference in aDNA preservation. Further, given the high sample dropout in Lake Victoria and, to some degree, Lake Chala, future studies may need to process a large number of samples to accumulate a sufficient number that yield endogenous aDNA. Methods predicting aDNA preservation from a sample's characteristics (Collins et al., 2009; Scorrano et al., 2015) or that detect endogenous DNA as early as possible in the process from DNA extraction to sequencing (Enk et al., 2013; Wales et al., 2012) may help to focus efforts on the most promising samples. Given the often-low endogenous content of samples, the application of hybridization sequence capture can significantly improve data yield and may be a necessary but powerful step for samples from poorly-preserving sites such as Lake Victoria, especially when targeting nuclear loci. Co-extraction of inhibitory substances like humic acids or metal oxides can hamper enzymatic steps during library preparation. Further purification of extracts or the use of enzymes that are more resilient to such inhibition could be potential remedies (King et al., 2009).

The aDNA preserved in fossil material from tropical regions is of particular interest to evolutionary biologists because the tropics harbour more biodiversity than temperate or arctic regions (Jablonski et al., 2006; Rolland et al., 2014) and are home to some textbook examples of adaptive radiation, such as lacustrine cichlid fishes. Our study demonstrates that it is possible to generate ancient DNA data from fish fossils found in the bottom sediments of tropical lakes, which can be used for taxonomic assignment and phylogenetic analysis. Future studies could use such data to reveal temporal changes in community composition, genetic diversity and population connectivity and continuity and deliver fundamental new insights into evolutionary and ecological processes in these ecosystems. Baselines of past genetic diversity may also help to define conservation goals. Freshwater diversity is under pressure worldwide (Reid et al., 2019), including the cichlid fishes of the East African lakes (Kishe-Machumu et al., 2018). Over the last century, and especially since the 1950s, *Oreochromis* species, and other fish taxa likely unintentionally with them, have been translocated to many natural and artificial water bodies across Africa for aquaculture and fisheries purposes (Pullin & Lowe-McConnell, 1982; Shechonge et al., 2019; Tibihika et al., 2020), and have often introgressed into native populations and species (Blackwell et al., 2021; Ndiwa et al., 2014; Shechonge, 2018). This makes it increasingly challenging to document the natural distribution of species and the natural genetic diversity and uniqueness of populations. Threats to this diversity can potentially be better understood and addressed by conservation efforts if they are guided by information on distribution ranges and genetic diversity predating the Anthropocene (Izzo et al., 2016; Kemp & Hadly, 2016).

Tropical lake sediments have already provided a plethora of information on past climate conditions, changes in the composition of terrestrial vegetation and long-term aquatic ecosystem dynamics, using a multitude of macroscopic, microscopic, isotopic and molecular proxies. The successful generation of genetic data from lake sediment-embedded fish remains opens up many new possibilities to investigate evolutionary processes and tropical ecosystem dynamics (Cohen, 2012; Cuenca-Cambronero et al., 2022).

AUTHOR CONTRIBUTIONS

MM initiated the study, helped acquire funding, guided the processing of all samples except Kivu 1 and 4, prepared samples for dating, led the 2018 coring campaign on Lake Victoria, analysed sequence data and wrote the manuscript with input from co-authors. OS and DV co-edited the manuscript, and SH, NW, WS and ML provided comments. EJ and NL processed Lake Victoria fossils. SH processed sample Kivu 1, together with MM. NW processed sample Kivu 4 and provided sequences. MK and SM co-organized and participated in sediment coring on Lake Victoria. JD and DV contributed samples Chala 1–10. ML provided laboratory space. WS contributed to

conceptualizing the research approach. OS acquired funding, initiated and facilitated the study.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

Short read data generated for this study is available for download from the European Nucleotide Archive ENA (https://www.ebi.ac.uk/ena/, project accession PRJEB61301).

BENEFIT-SHARING

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