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Lake Tanganyika as an evolutionary reservoir of old lineages of East African cichlid fishes: Inferences from allozyme data

M. Nishida

Department of Marine Sciences, University of the Ryukyus, Nishihara, Okinawa 903-01 (Japan)
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Abstract. Genetic differences between 20 species of cichlid fish, representing all the 12 tribes proposed for the cichlid fish fauna of Lake Tanganyika, were studied by allozyme electrophoresis. Most species were genetically very differentiated from each other. Phylogenetic analysis based on the allozyme data indicated that at least seven old, ancestral lineages have contributed to the present cichlid fauna of the lake. Lake Tanganyika, the oldest of the rift-valley lakes, can be recognized as an evolutionary reservoir of major lineages of cichlids in East Africa.
Key words. Cichlid fish; Lake Tanganyika; allozyme; genetic difference; phylogenetic relationship.

The presence of vast numbers of endemic species belonging to a single fish family, the Cichlidae, within each African rift-valley lake presents a unique challenge to evolutionary biologists[1–3]. Though considerable attention has been directed to the phenomenon, the general phylogenetic framework of these fishes has yet to be determined, primarily because of the difficulty of identifying morphological features for phylogenetic analysis.

Most cichlid species in Lakes Victoria and Malawi have been regarded as belonging to a single genus, *Haplochromis*, or being closely related to this genus[2]. Recently, Meyer et al.[4] examined mitochondrial DNA differences among representative cichlids in Lake Victoria and some in Lakes Malawi and Tanganyika. Lake Victoria cichlids were shown to be genetically quite similar to each other and more akin to those from Lake Malawi than

those from Lake Tanganyika. Cichlids from Lake Malawi were also more closely related to each other than to any non-Malawi species studied.

In contrast to the cichlids of Lakes Victoria and Malawi, those of Lake Tanganyika were recognized by early researchers [5-7] to belong to many different genera, because of their considerable diversification, particularly in morphological features [2,8]. Poll [9] has recently proposed 12 tribes of Tanganyika cichlids. To clarify the genetic and phylogenetic basis of their large diversification in morphology and taxonomy, I examined allozyme variation in 20 species from Lake Tanganyika, representing 20 (36%) genera and all of the 12 tribes proposed by Poll [9]. The usefulness of allozyme analysis for genetic and phylogenetic investigations has been demonstrated in numerous studies [10-12].

Materials and methods

Samples. Specimens were collected using gill nets or a seine, or from local fisherman, at three locations in the northwestern part of Lake Tanganyika: Ruzizi, including the Kilomoni and Uvira coasts (R), Luhanga (L), and Bemba (B). For each species, specimens collected from a single locality were used for analysis. The tribes (with their code number) and species (species and locality abbreviations and number of individuals) examined are as follow: 1) Tilapiini – *Boulengerochromis microlepis* (BOMI, R, 18), *Oreochromis niloticus* (ORNI, R, 25); 2) Haplochromini – *Astatotilapia burtoni* (ASBU, R, 12); 3) Tylochromini – *Tylochromis polylepis* (TYPO, R, 17); 4) Lamprologini – *Neolamprologus brichardi* (NEBR, L, 43), *Telmatochromis temporalis* (TETE, L, 8), *Julidochromis marlieri* (JUMA, L, 6); 5) Tropheini – *Tropheus moorii* (TRMO, L, 93), *Simochromis babaulti* (SIBA, R, 11), *Petrochromis trewavasae* (PETR, L, 10); 6) Eretmodini – *Eretmodus cyanostictus* (ERCY, B, 12); 7) Ectodini – *Ectodus descampsi* (ECDE, R, 9), *Lestradea perspicax* (LEPE, B, 16), *Grammatotria lemairii* (GRLE, R, 8); 8) Trematocarini – *Trematocara nigrifrons* (TRNI, R, 10); 9) Bathybatini – *Bathybates graueri* (BAGR, R, 10); 10) Limnochromini – *Limnochromis auritus* (LIAU, R, 2); 11) Cyprichromini – *Cyprichromis microlepidotus* (CYMI, L, 10); 12) Perissodini – *Perissodus microlepis* (PEMI, L, 21), *Plecodus straeleni* (PLST, B, 2). Voucher specimens have been registered and deposited in the collection of the Department of Marine Sciences, University of the Ryukyus.

Electrophoresis. Tissue extracts of eye, liver and skeletal muscle were prepared from each specimen. Twenty-one enzyme loci were analyzed by starch gel electrophoresis, using the method previously described [13], essentially according to standard procedures [14-16]. The following enzymes (EC numbers and loci) were examined: aspartate aminotransferase (EC 2.6.1.1; *Aat-1*, *Aat-2*, *Aat-3*), alcohol dehydrogenase (EC 1.1.1.1; *Adh*), aconitate hydratase (EC 4.2.1.3; *Ah*), creatine kinase (EC 2.7.3.2; *Ck-2*), fructose-1,6-diphosphatase (EC 3.1.3.11; *Fdp-2*), fumarate hydratase (EC 4.2.1.2; *Fh*), glucosephosphate isomerase (EC 5.3.1.9; *Gpi-1*, *Gpi-2*), isocitrate dehydrogenase (EC 1.1.1.42; *Idh-1*, *Idh-2*), lactate dehydrogenase (EC 1.1.1.27; *Ldh-1*, *Ldh-3*), malate dehydrogenase (EC 1.1.1.37 *Mdh-1*, *Mdh-2*, *Mdh-3*), mannosephosphate isomerase (EC 5.3.1.8; *Mpi*), phosphoglucose dehydrogenase (EC 1.1.1.44; *Pgdh*), phosphoglucomutase (EC 2.7.5.1; *Pgm*), and superoxide dismutase (EC 1.15.1.1; *Sod*).

Data analysis. From individual genotypes, allele frequencies were determined for all species, and based on these, the genetic distances of Nei [17] (D_N) and Rogers [18] (D_R) were calculated. Though D_N is not a metric, in contrast to D_R, it is a measure of codon substitution per locus, and may possibly be linearly correlated to time.

The matrix of D_N was used to construct an UPGMA [19] (unweighted pair-group method using arithmetic averages) tree. UPGMA was originally developed as a phenetic clustering method, but may provide a good evolutionary tree [20-22], assuming that the rate of molecular evolution is approximately constant [22-24] and the genetic distance measure used is proportional to evolutionary change as in D_N.

The molecular clock, however, may not be applicable and consequently, a kind of minimum evolution tree by the neighbor-joining (NJ) method [25] and character-state Wagner parsimony trees by the PAUP algorithm [26], were also constructed to examine phylogenetic relationships among species. In neither of these methods is the rate of evolution assumed to be constant. The D_R matrix was used for NJ analysis. In the PAUP analysis, loci were considered as characters and alleles as unordered character states. For each locus, the state assigned was that of the most common allele. MULPARS/GLOBAL SWAP options were used in this analysis. Undirected trees from these analyses were rooted with *Tylochromis* as an outgroup. The genus is suggested to represent the sister group of the remaining African lineage by Stiassny [27].

Results

The allele frequencies at 21 loci in 20 species are listed in table 1. All 21 loci scored were variable for species examined. Nei's and Rogers' genetic distance measures among the 20 species are shown in table 2. Most species showed considerable genetic divergence from one another with the exception of *Lestradea*, which was quite close genetically to *Ectodus*. Most D_N values considerably exceeded those observed in cichlids of Lakes Victoria [28,29] and Malawi [30].

The UPGMA tree derived from D_N is presented in figure 1a. Seven lineages with high divergence points at around $D_N = 1.0$ or more were apparent in the tree. Of these, six were represented only by species of a single tribe, while the remaining one consisted of many species from various tribes. (For convenience, this large lineage

is hereafter referred to as the H-lineage since it includes the haplochromine lineage).

The seven lineages were also recognized in an NJ tree (fig. 2), though the banching order among the lineages and within the H-lineage differed somewhat from that in the UPGMA tree.

Character-state Wagner parsimony analysis consistently supported the seven major lineages observed in the distance analysis. Using the PAUP algorithm, the 90 equally most parsimonious trees were obtained (each with 116 steps, consistency index CI = 0.69). These trees differed only in the arrangement of the major lineages or species within the H-lineage. A strict consensus tree from all 90 Wagner parsimony trees as well as the UPGMA and NJ trees was obtained using the CONTREE subroutine in the PAUP program, and is shown in figure 3.

Discussion

The present results indicate that there are many – at least seven – old lineages in the cichlid fauna of Lake Tanganyika, and that one of them, the H-lineage, is comprised of various groups of cichlids. This finding is supported by the basic agreement of the results of analysis by three different phylogenetic methods. This agreement in-

Table 1. Allele frequencies, expressed as percentages, at 21 genetic loci in 20 cichlid fishes of Lake Tanganyika. Species abbreviations are as in text. Alleles are designated alphabetically in the order of decreasing anodal mobility of their protein products. Where the allele frequencies are not given, the frequency is 100.

Locus	Species examined BOMI	ORNI	ASBU	TYPO	NEBR	TETE	JUMA	TRMO	SIBA	PETR
Aat-1	b	d	b	b	d	d(67) e(33)	d	b	b	b
Aat-2	e	a(07) c(89) e(04)	c(04) e(96)	b	e(01) g(95) i(03)	g	g	e	c	e
Aat-3	d	d	c	f	c	e	e	c	c	e
Adh	d	g(63) h(37)	f	f	c(99) d(01)	e(81) f(19)	a	d	f	f
Ah	e	c(07) e(75) g(14) i(04)	g	a	e(98) g(02)	e	e(83) g(17)	e(02) g(92) i(06)	g	g
Ck-2	e	b(98) c(02)	c	f	a	a	a	c	c(82) e(18)	c
Fdp-2	g(94) h(06)	f	a(04) c(04) d(92)	b	a	a	a	d(61) e(39)	c(05) d(95)	d(30) e(70)
Fh	c	b(60) c(40)	b	c	b	b	–	c	c	c
Gpi-1	f(97) m(03)	g(90) h(10)	h	f	h(07) k(22) l(20) m(50) n(01)	m	m	c	c	c(90) f(10)
Gpi-2	g	b	b(96) e(04)	e	b(98) c(02)	a(13) b(88)	b	b	b	b
Idh-1	a(81) c(19)	b(06) c(94)	e	a(09) b(29) c(62)	e(08) g(92)	g	g	c(99) e(01)	c	c
Idh-2	b	b	b	d(09) e(91)	b	b	b	e(99) f(01)	b	d
Ldh-1	a	b	b	c	b	b	b	b	b	b
Ldh-3	e(03) f(97)	e	b	e	c	c	c	b	b	b(39) c(61)
Mdh-1	g	d(16) e(84)	b(04) d(96)	b	a(02) b(07) d(87) f(03)	d	d	d	d	d(61) f(39)
Mdh-2	c	a	d	d	d(99) f(01)	d	d	d	d	d
Mdh-3	b	a(92) c(08)	c	b	a	c	a	c	c	c
Mpi	b	b	a(06) b(94)	c	b	a(08) b(92)	a	b	b	b
Pgdh	e	f	f	a	c(41) d(57) e(02)	c(19) d(81)	c(33) d(67)	c	e	e
Pgm	i	b	d	e	a(01) d(99)	b(13) d(68) f(13)	d	d	d	d
Sod	d	d(78) g(22)	c	d	a	d	d	c	c	c

Table 1. continued.

	ERCY	ECDE	LEPE	GRLE	TRNI	BAGR	LIAU	CYMI	PEMI	PLST
Aat-1	b	b	b(97) d(03)	b	d	d	b	b	b	b
Aat-2	f	c(06) e(94)	c(03) e(97)	e	h(95) j(05)	j	h	h	h(60) i(40)	i
Aat-3	c	c	c	c	d	c	c	c	c	c
Adh	f(96) h(04)	f(11) h(89)	h	h(94) i(06)	–	d	h	h	f	f
Ah	e(33) g(61)	f(28) g(67) h(06)	g	f(29) g(71)	c	e	h	c(10) e(85) g(05)	g	g
Ck-2	b(09) c(91)	f	f	g	e(70) f(30)	f	e	f	f	f
Fdp-2	c	c	a(09) c(88) d(03)	c(88) e(13)	d	f	c	c	c	c
Fh	b	b	b	b	b	c	b	b	b	b
Gpi-1	f(33) h(67)	c(94) e(06)	c(94) e(06)	c	j	f	d(25) f(75)	c	c(05) e(95)	c(25) e(75)
Gpi-2	a	b(22) e(50) g(28)	b(63) e(28) g(09)	e	a	a(65) b(35)	a	b	b	b
Idh-1	c	e	e	c(83) d(17)	c	c	g	c	c(06) e(94)	c
Idh-2	d	b	b	b	a	a	b	c	b	b
Ldh-1	b	b	b	b	c	c(20) d(80)	b	b	b	b
Ldh-3	e	e	e	g	c(45) e(55)	e	e	c	e	e
Mdh-1	f	c(11) d(89)	d	d	c	d	d	d	d	d
Mdh-2	d	d(94) f(06)	d	d	d	d	d	d	d	d
Mdh-3	c	a	a	a	a	c	c	c	c	c
Mpi	b	c	c	b(40) c(60)	–	–	–	a(70) b(30)	b	–
Pgdh	d	d	d	d	d	b(95) e(05)	d	b	b	a
Pgm	d	b(11) d(89)	d(97) g(03)	i	e	e(95) f(05)	d	d	d	d
Sod	d(38) f(62)	d	d	d	e(75) g(25)	d	d	b	d	d

Table 2. Nei's[17] (above diagonal) and Rogers'[18] (below diagonal) genetic distances between 20 cichlid fishes of Lake Tanganyika. For species abbreviations, see text.

	BOMI	ORNI	ASBU	TYPO	NEBR	TETE	JUMA	TRMO	SIBA	PETR	ERCY	ECDE	LEPE	GRLE	TRNI	BAGR	LIAU	CYMI	PEMI	PLST
BOMI	–	1.32	1.66	1.37	1.88	1.61	1.91	1.36	1.34	1.33	1.81	1.53	1.60	1.28	2.24	1.30	1.41	2.16	1.62	1.80
ORNI	0.73	–	1.16	1.94	0.93	0.98	1.04	1.40	1.07	1.38	1.24	1.08	1.05	1.12	1.20	1.03	1.30	1.33	1.04	1.01
ASBU	0.80	0.69	–	1.89	0.80	0.83	1.14	0.35	0.28	0.42	0.58	0.61	0.56	0.79	1.82	1.50	0.78	0.78	0.39	0.49
TYPO	0.74	0.84	0.84	–	2.92	2.19	2.26	1.50	1.49	1.42	1.30	1.09	1.17	1.35	1.39	0.90	1.41	1.72	1.21	0.93
NEBR	0.83	0.61	0.55	0.92	–	0.25	0.25	1.00	0.93	1.14	1.02	0.81	0.75	0.90	1.24	1.27	0.81	0.79	0.79	0.87
TETE	0.78	0.64	0.57	0.87	0.27	–	0.16	1.08	0.95	0.89	0.94	0.93	0.87	0.98	1.46	1.12	0.67	0.81	0.71	0.76
JUMA	0.85	0.64	0.68	0.88	0.25	0.19	–	1.25	1.14	1.08	1.44	0.87	0.80	1.02	1.41	1.25	0.87	0.95	0.99	0.94
TRMO	0.74	0.75	0.31	0.77	0.63	0.66	0.72	–	0.23	0.26	0.74	0.84	0.76	0.80	1.93	0.96	1.02	0.67	0.70	0.65
SIBA	0.74	0.66	0.25	0.77	0.60	0.62	0.68	0.22	–	0.22	0.57	0.98	0.90	0.94	1.91	1.30	1.20	0.74	0.71	0.65
PETR	0.73	0.74	0.37	0.75	0.67	0.61	0.67	0.26	0.24	–	0.65	0.81	0.75	0.80	1.77	1.10	0.87	0.67	0.52	0.46
ERCY	0.84	0.70	0.46	0.73	0.64	0.63	0.76	0.52	0.45	0.49	–	0.67	0.69	0.77	1.14	1.02	0.48	0.72	0.50	0.46
ECDE	0.77	0.67	0.48	0.66	0.56	0.60	0.60	0.58	0.63	0.57	0.50	–	0.01	0.24	1.26	1.14	0.39	0.59	0.35	0.36
LEPE	0.79	0.67	0.44	0.69	0.54	0.59	0.57	0.54	0.60	0.53	0.51	0.06	–	0.26	1.28	1.11	0.41	0.56	0.33	0.34
GRLE	0.72	0.69	0.56	0.74	0.60	0.62	0.64	0.56	0.63	0.56	0.55	0.25	0.27	–	1.30	1.37	0.57	0.70	0.68	0.59
TRNI	0.88	0.70	0.82	0.75	0.71	0.77	0.75	0.84	0.83	0.82	0.67	0.71	0.72	0.72	–	0.97	1.07	1.33	1.64	1.54
BAGR	0.73	0.66	0.77	0.60	0.71	0.67	0.71	0.62	0.73	0.67	0.65	0.67	0.66	0.74	0.64	–	0.96	0.86	0.83	0.83
LIAU	0.75	0.73	0.54	0.75	0.56	0.50	0.59	0.64	0.70	0.58	0.40	0.35	0.34	0.45	0.66	0.62	–	0.58	0.43	0.49
CYMI	0.89	0.74	0.55	0.82	0.55	0.57	0.61	0.50	0.54	0.50	0.52	0.47	0.44	0.51	0.74	0.59	0.44	–	0.44	0.47
PEMI	0.79	0.65	0.33	0.70	0.55	0.52	0.63	0.51	0.52	0.41	0.41	0.33	0.30	0.51	0.81	0.57	0.36	0.38	–	0.12
PLST	0.83	0.65	0.40	0.61	0.58	0.55	0.61	0.49	0.49	0.38	0.39	0.34	0.31	0.47	0.79	0.57	0.39	0.38	0.14	–

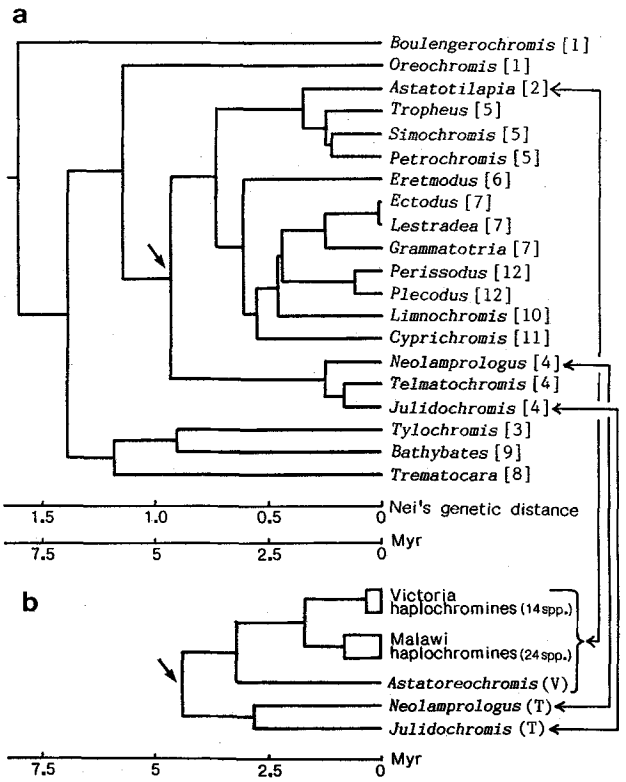

Figure 1. *a* A UPGMA tree derived from Nei's genetic distances between 20 cichlid fishes of Lake Tanganyika. The numeral after a genus name is the code number of tribes as shown in text. For the time scale, see 'Discussion' in text. *b* An evolutionary tree based on mitochondrial DNA data from cichlids in Lakes Victoria (V), Malawi and Tanganyika (T) (after Meyer et al.[4]). Arrows denote corresponding nodes or branches between the two trees.

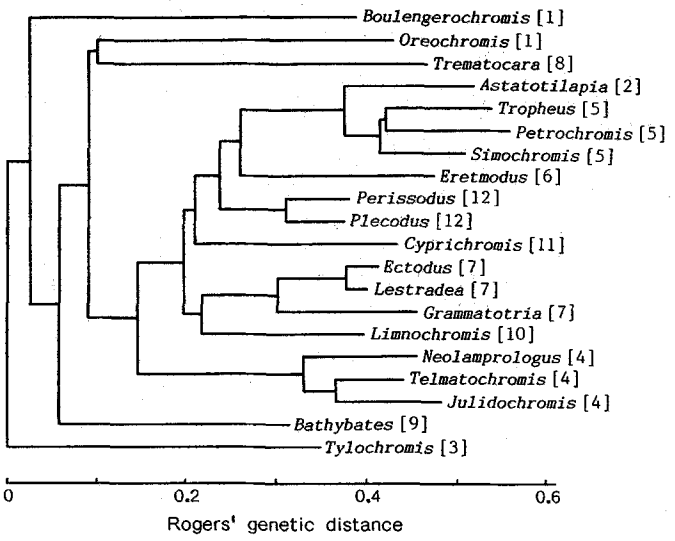

Figure 2. A neighbor-joining tree derived from Rogers' genetic distances between 20 cichlid fishes of Lake Tanganyika. The tree is rooted with *Tylochromis* as an outgroup. The numeral after a genus name is the code number of tribes as shown in text.

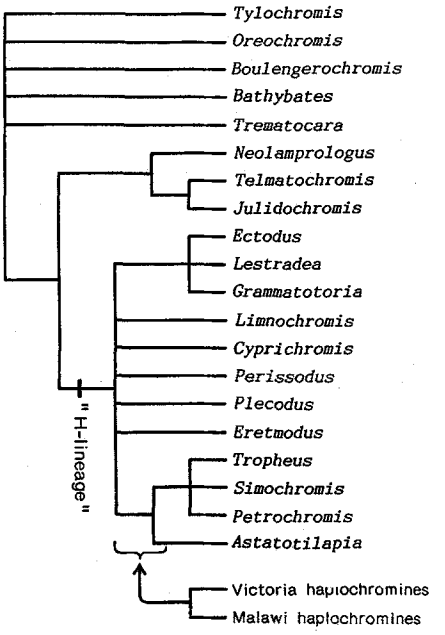

Figure 3. A strict consensus tree for 20 cichlid fishes of Lake Tanganyika based on the 90 equally most parsimonious trees generated from character-state Wagner parsimony analysis by PAUP and UPGMA and NJ trees in figures 1 and 2. The arrow shows the presumed place at which the haplochromine lineage of Lake Victoria and Lake Malawi is connected to the consensus tree of Tanganyika cichlids.

dicates the possibility that there has been no considerable variation in the rates of allozyme evolution among lineages of these fishes.

Assuming an approximately constant rate of allozyme evolution, the time of divergence can be estimated by calibrating D_N in two ways, empirical and theoretical. In the present analysis, the former method was employed, using time estimation from mitochondrial DNA (mtDNA) divergence by Meyer et al.[4] for comparison. They used the same species or groups as in the present study (*Neolamprologus brichardi* and *Julidochromis* in Lamprologini as well as many haplochromines in Haplochromini) and estimated the divergence time between Lamprologini and Haplochromini as being about 4.5 million years ago based on mtDNA data (fig. 1 b). Here, the D_N value between these two lineages was approximately 0.9 (fig. 1 a), which gave a calibration of $1D_N = 5$ Myr. This result is identical to the theoretical calibration proposed by Nei[20]. Though Nei's formula gives a time less than that obtained by the empirical method in other studies[31,32], close agreement with the time estimation from mtDNA shows this formula to be applicable to time estimation from the present data as a first approximation. The time scale in figure 1 a was based on this formula.

The seven lineages observed in cichlids of Lake Tanganyika appear to be quite old, as much as 5 million years or more, based on the above time estimation (fig. 1 a). Lake Tanganyika has been estimated to have been

formed about 2 million years ago[8, 33]. These lineages may thus possibly have already been in existence before its formation. The tribes Tylochromini, Tilapiini, Lamprologini and Haplochromini are not endemic to Lake Tanganyika, and their ancestors have been suggested to have contributed independently to the cichlid fauna of this lake[2, 6, 34]. It is apparent from this study that there are at least three further lineages as probable ancestral groups for the fauna. I conclude that the cichlid flock of Lake Tanganyika is a polyphyletic conglomerate with many ancestral lineages.

The evolutionary tree mainly for Victoria and Malawi cichlids by Meyer et al.[4] (fig. 1 b) can be superimposed over the present one for Tanganyika cichlids (fig. 1 a) using the shared time framework based on commonly examined groups. In this manner, a composite tree (fig. 3) can be obtained, whose branch of haplochromines in Lake Victoria and Lake Malawi examined by Meyer et al.[4] is only a part of that of the H-lineage found in Tanganyika cichlids.

It is of interest that the H-lineage also leads many groups of Lake Tanganyika. More than half the tribes proposed by Poll[9] are in this lineage. Some branches in this lineage in Lake Tanganyika may possibly be shared by the cichlid fauna in Lake Malawi[35, 36]. Extensive inter-lacustrine genetic studies should be conducted to fully examine the validity of the proposed tribes, particularly those in the H-lineage, in the inter-lacustrine perspective.

From the present finding of many old lineages, Lake Tanganyika, the oldest among the rift-valley lakes[33], appears to have inherited its major lineages of cichlids from various ancestral lakes that once existed in the area. Indeed, multiple origins has been suggested for the present Lake Tanganyika[33]. The lake may be concluded to have served as an evolutionary reservoir of old ancestral lineages of East African cichlids. Diverse cichlid groups in this lake should provide useful data for reconstructing the fundamental phylogeny of East African cichlids, which is essential to a more detailed study of the evolution of these fishes.

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