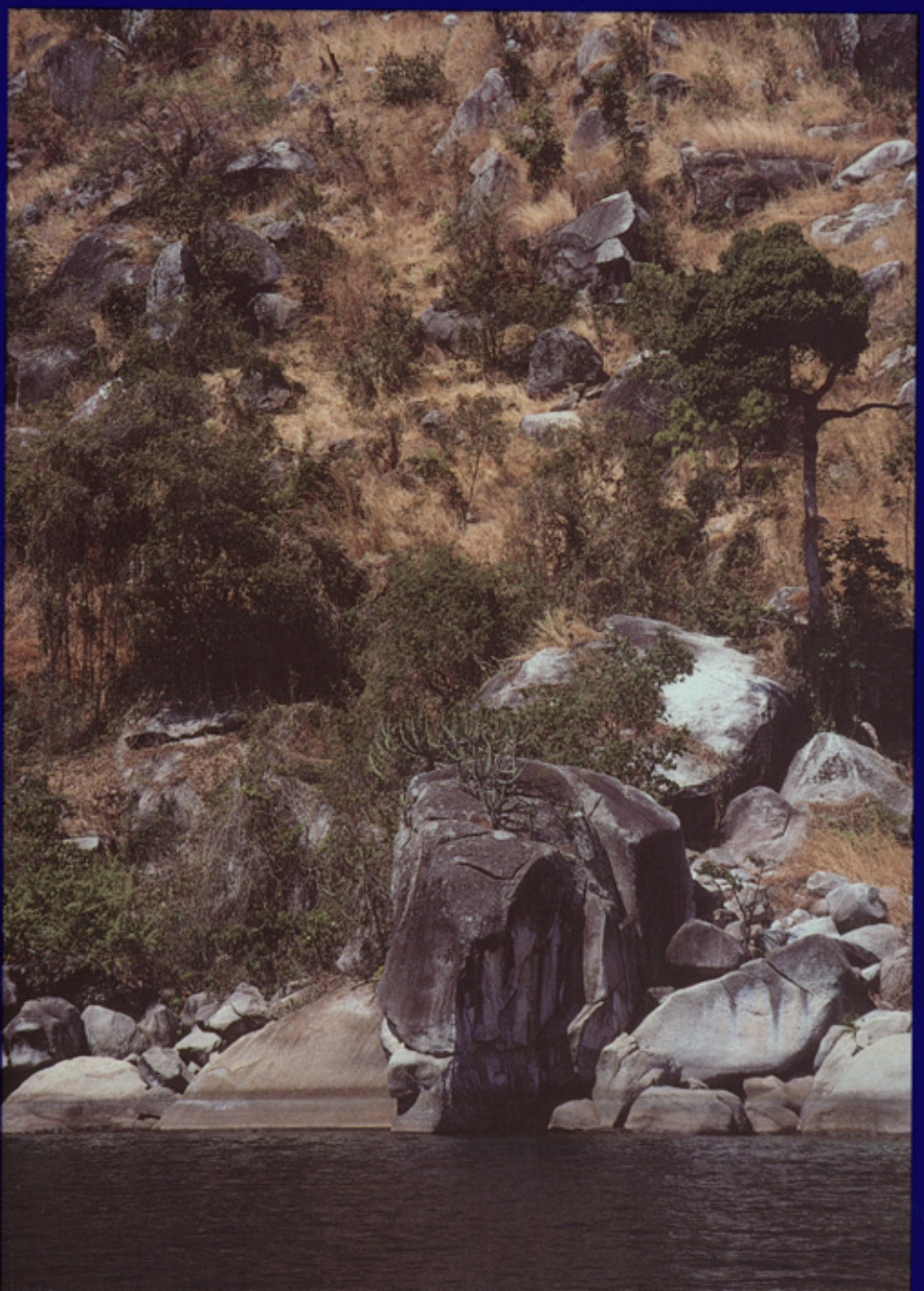
ISSN 0962-1083

VOLUME 10
NUMBER 5
MAY
2001

MOLECULAR ECOLOGY



Published by
Blackwell Science

Molecular Ecology (2001) **10**, 1207–1225

Population structure in two sympatric species of the Lake Tanganyika cichlid tribe Eretmodini: evidence for introgression

LUKAS RÜBER,*†¶ AXEL MEYER,‡ CHRISTIAN STURMBAUER§ and ERIK VERHEYEN†
*Zoological Museum, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland, †Section of Taxonomy and Biochemical Systematics, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium, ‡Department of Biology, University of Konstanz, 78457 Konstanz, Germany, §Department of Zoology and Limnology, University at Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria

Abstract

Patterns of genetic differentiation were analysed and compared in two sympatric species of the endemic Lake Tanganyika cichlid tribe Eretmodini by means of mitochondrial DNA (mtDNA) sequences of the control region and six microsatellite DNA loci. The sample area covers a total of 138 km of mostly uninterrupted rocky shoreline in the Democratic Republic of Congo and includes the entire distribution range of *Tanganicodus* cf. *irsacae* that stretches over a distance of 35 km. Both markers detected significant genetic differentiation within and between the two species. *T.* cf. *irsacae* contained lower overall genetic variation than *Eretmodus cyanostictus*, possibly due to its more restricted range of distribution and its smaller effective population sizes. Complete fixation of *Tanganicodus* mtDNA haplotypes was observed in *Eretmodus* at two localities, while at two other localities some *Tanganicodus* individuals possessed *Eretmodus* mtDNA haplotypes. Taking into account the relatively large average sequence divergence of 6.2% between the two species, as well as the geographical distribution of mtDNA haplotypes in the lake, the observed pattern is more likely to be a consequence of asymmetric introgression than of shared ancestral polymorphism. As there is significant population differentiation between sympatric *Tanganicodus* and *Eretmodus* populations, the events of introgressions may have happened after secondary contact, but our data provide no evidence for ongoing gene flow and suggest that both species are reproductively isolated at present time.

Keywords: genetic diversity, hybridization, microsatellite DNA, mitochondrial DNA

Received 18 August 2000; revision received 23 November 2000; accepted 23 November 2000

Introduction

The cichlid species flocks of the East African Great Lakes provide excellent opportunities to study speciation and adaptive radiation (Fryer & Illes 1972; Meyer 1993). Several cichlid lineages show unique levels of ecological diversification and species packing, and exceptionally fast speciation rates have been reported for the haplochromine lineages in lakes Malawi and Victoria (Seehausen *et al*. 1999). Each species flock is believed to have evolved independently from different ancestral riverine stocks via intralacustrine speciation (Meyer 1993). As lakes Tanganyika, Malawi, and Victoria differ widely in their relative ages, they offer a unique opportunity to study different stages of adaptive radiations in cichlids and to detect general and unique characteristics of their species flocks (Sturmbauer 1998).

Among several potential mechanisms promoting speciation in lacustrine cichlids, disruptive sexual selection on polymorphic male colouration has been suggested to play an important role during sympatric speciation in polygynous, maternal mouthbrooders, such as the haplochromines from lakes Malawi and Victoria (Seehausen *et al*. 1999). Other studies indicate that selection for ecological divergence may also be a major driving force of cichlid speciation (e.g. Schliewen *et al*. 1994). The evolution of divergent phenotypes and resource use may precede or initiate the speciation process, or it may evolve after reproductive isolation via disruptive sexual selection has occurred, and thus would facilitate the coexistence of species (Galis & Metz 1998). Other hypotheses for speciation in rock-dwelling cichlids emphasize the importance of extrinsic factors for speciation, such as lake level fluctuations

Correspondence: L. Rüber. ¶Present address: Museo Nacional de Ciencias Naturales, José Gutiérrez Abascal 2, 28006 Madrid, Spain. E-mail: ruber@mncn.csic.es

promoting geographical isolation (Fryer & Illes 1972; Ribbink *et al*. 1983).

With an estimated age of 9–12 Myr Lake Tanganyika is the oldest of the East African Rift Lakes (Cohen *et al*. 1993) harbouring approximately 200 endemic cichlid species. In sharp contrast to the species flocks of haplochromine cichlids of lakes Malawi and Victoria, Lake Tanganyika contains several evolutionary lineages that probably predate the lake's ancient history (Nishida 1991; Sturmbauer & Meyer 1993). Past lake level drops, some of which have split the lake up into isolated subbasins, have strongly affected rocky habitat patches in the littoral of Lake Tanganyika (Cohen *et al*. 1997). The effects of such changes in water level, at different temporal and spatial scales, were early hypothesized by Brooks (1950) and Poll (1952) to be one of the underlying causes of the faunal diversity found in Lake Tanganyika (but see Snoeks *et al*. 1994). Recently, molecular studies confirmed that these major vicariant events strongly influenced the phylogeographic patterns in some rock-dwelling cichlids (Sturmbauer & Meyer 1992; Sturmbauer *et al*. 1997, 2001; Rüber *et al*. 1999; but see Meyer *et al*. 1996). In Lake Malawi, which has persisted as a single basin throughout its history, several haplochromine species exhibit high levels of population subdivision, which points to the importance of allopatric divergence among habitat patches for speciation of the stenotopic *mbuna* cichlids that do not disperse over areas of deep water and/or long stretches of sandy shoreline (van Oppen *et al*. 1997a; Arnegard *et al*. 1999; Markert *et al*. 1999). The existence of concordant patterns of population structure across sympatric taxa would be a strong indication of the importance of shared historical factors for shaping population demography. On the other hand, noncongruent patterns may help to identify important differences among taxa concerning their dispersal abilities and habitat requirements that are relevant for past demographic events and evolutionary processes.

Cichlids from the tribe Eretmodini are endemic to Lake Tanganyika and occur along shallow rocky and pebble shorelines, mostly at depths lower than 5 m. A recent phylogenetic analysis based on mitochondrial DNA (mtDNA) sequences identified six distinct eretmodine lineages which are not concordant with the current taxonomy and suggest the 'existence' of cryptic species (Rüber *et al*. 1999). Based on the mtDNA phylogeny, the genera *Eretmodus*, *Spathodus*, and *Tanganicodus* are considered nonmonophyletic and are thus in need of a taxonomic reassessment. A lake-wide phylogeographic study demonstrated a high degree of intralacustrine endemism of the eretmodine lineages (Rüber *et al*. 1999). It was suggested that both the patchy distribution of rocky shorelines and Pleistocene lake level fluctuations have significantly influenced distribution patterns in these cichlids. Interestingly, similar trophic phenotypes assigned to different genetic lineages generally show complementary distributions. This observation was taken as evidence that species interactions might have also played a determinant role in shaping their present distribution (Rüber *et al*. 1999).

The two nominal species *Eretmodus cyanostictus* and *Tanganicodus* cf. *irsacae* (defined as mtDNA lineage C and D in Rüber *et al*. 1999) are easily distinguishable by differences in their body shape, dentition and colouration (Huysseune *et al*. 1999; Rüber *et al*. 1999; Rüber & Adams 2001). *Eretmodus* is an algae scraper, whereas *Tanganicodus* is a specialized invertebrate picker (Yamaoka *et al*. 1986). The two species are monogamous, sexually monochromatic, biparental mouthbrooders that perform a female-to-male shift of the hatchlings during mouthbrooding (Kuwamura *et al*. 1989). *T.* cf. *irsacae* has the most narrow distribution of all eretmodine cichlids and is only known from four localities over a distance of about 35 km in the south-western part of Lake Tanganyika in the Democratic Republic of Congo (Fig. 1). *E. cyanostictus*, on the other hand, has a much wider distribution range that covers about 600 km of coastline in the southern part of the lake.

In this paper we characterize the patterns of genetic variation in these two cichlid species, at localities where they are sympatric, using microsatellite markers and sequences of the mtDNA control region. The objectives of this study are: (i) to examine differences in genetic diversity and population structure that may be related to differences in distribution ranges; and (ii) to investigate the possibility of introgression between these closely related species under sympatric conditions.

Material and methods

Sample collection

A total of 123 specimens of *Eretmodus cyanostictus* were collected from five localities covering 138 km of mostly uninterrupted rocky shoreline, and 75 specimens from *Tanganicodus* cf. *irsacae* were collected from four localities in the Democratic Republic of Congo (Fig. 1), covering its entire known distribution range of 35 km. This study includes two populations of *Eretmodus* (KOR and KAP; for locality abbreviations see Fig. 1) that were not studied in a previous phylogenetic analysis (Rüber *et al*. 1999). For the microsatellite analyses two populations (*Eretmodus* KAP and *Tanganicodus* KIK) were omitted due to small sample sizes, therefore, a total of 117 specimens of *E. cyanostictus* and 69 specimens of *T.* cf. *irsacae* were used. Samples were preserved in 70% ethanol and voucher specimens have been deposited in the Africa Museum in Tervuren, Belgium.

Characterization of microsatellite DNA

DNA was extracted using a proteinase K, phenol–chloroform protocol (Kocher *et al*. 1989). A total of 10 microsatellite

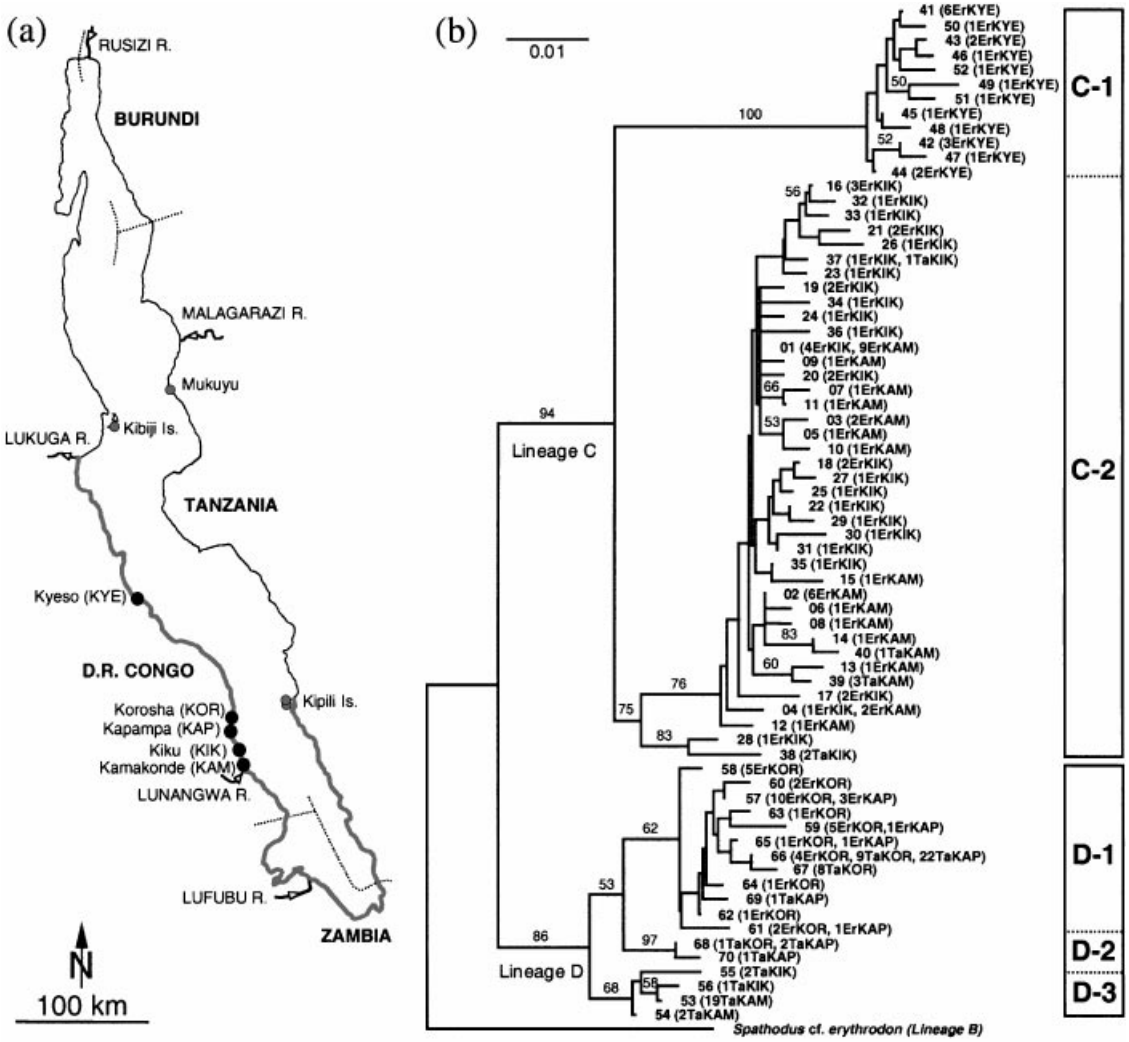


Fig. 1 (a) Map of Lake Tanganyika with localities from where fish were collected for this study. Locality abbreviation: Kyeso (KYE), Kiku (KIK), Kapampa (KAP), Korosha (KOR), and Kamakonde (KAM). Known distribution of *Eretmodus cyanostictus* is indicated in grey. The distribution of *Tanganicodus* cf. *irsacae* is limited to the coastline between KIK and KAM. (b) Neighbour-joining tree based on partial control region sequences of the 70 haplotypes found among 198 sequenced specimens. Haplotype numbers (see Appendix II) are given next to each branch; in brackets, the number of individuals, species assignment (Er = *Eretmodus cyanostictus*; Ta = *Tanganicodus* cf. *irsacae*), and localities are given. The tree is rooted with *Spathodus* cf. *erythrodon*. Bootstrap percentage values based on 1000 replications are shown above branches. The assignments to the major genetic lineages (C and D) and clades are given in boxes.

loci, which were developed for other East African cichlid species, were initially tested by sequencing to assess their utility for population studies in eretmodine cichlids. For sequencing, polymerase chain reactions (PCR) were carried out in 25 μL volumes [Tris 10 mM, pH 9.0, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, and 0.65 units of *Taq* DNA polymerase (Pharmacia)] using the PCR conditions given in Kellog *et al*. (1995) for UNH001 and UNH002, Zardoya *et al*. (1996) for TmoM5, TmoM7, TmoM11, TmoM13, TmoM25, and TmoM27, and van Oppen *et al*. (1997b) for Pzeb1 and Pzeb3, with adjustments of annealing temperatures (Table 1). PCR fragments were either ligated into pGEM-T plasmid vectors (Promega) or using the TopoTA cloning kit (Invitrogene). Clones were sequenced with M13 universal (−40) and reverse primers with an Applied Biosystem 373 A DNA sequencer using the Taq Dye Deoxy Terminator Cycle Sequencing Kit FS (PE Biosystems).

Microsatellite markers

Once suitable microsatellite loci were selected (see Results) amplified products were electrophoresed on 6% denaturating polyacrylamide gels using an ALF Express DNA sequencer (Pharmacia) with short glass plates. Gels were run between 2–4 h, depending on the allele size, at 55 °C (15 W,

Table 1 Characterization of the cichlid microsatellite DNA loci examined for amplification in eretmodines. Underlined loci were selected for the population analysis

Locus	*Specimen*	Core sequence	*Length*	T_a (°C)	Reference
TmoM5*	*Tropheus moorii*	$(GC)_6(AC)_{14}$		55	Zardoya *et al*. (1996)
	Spathodus cf. *erythrodon* (B)	$(AC)_{33}$	314		(AJ407057)
	Eretmodus cyanostictus (C)	$(GC)_4(AC)_{13}$	282		(AJ407058)
TmoM11	*Tropheus moorii*	$(AC)_{19}$		50	Zardoya *et al*. (1996)
	Eretmodus cf. *cyanostictus* (A)	$(AC)_{16}$	169		(AJ407063)
	Eretmodus cyanostictus (C)	$(AC)_{10}$	157		(AJ407062)
	Tanganicodus cf. *irsacae* (E)	$(AC)_{20}$	177		(AJ407064)
TmoM25	*Tropheus moorii*	$(CA)_{23}$		50	Zardoya *et al*. (1996)
	Tanganicodus irsacae (A)	$(CA)_5CGT(CA)_{28}$	374		(AJ407069)
	Eretmodus cf. *cyanostictus* (A)	$(CA)_6CGT(CA)_{14}$	348		(AJ407068)
	Eretmodus cyanostictus (C)	$(CA)_5CGT(CA)_{12}$	342		(AJ407067)
	Tanganicodus cf. *irsacae* (E)	$(CA)_5CGT(CA)_{22}$	362		(AJ407070)
Pzeb1	*Pseudotropheus zebra*	$(GT)_{59}CC(T)_{16}$		55	van Oppen *et al*. (1997b)
	Eretmodus cyanostictus (C)	$(GT)_{17}GA(T)_{13}$	140		(AJ407052)
	Tanganicodus cf. *irsacae* (D)	$(GT)_7TTGC(T)_{15}$	124		(AJ407053)
Pzeb3	*Pseudotropheus zebra*	$(TG)_{12}$		55	van Oppen *et al*. (1997b)
	Eretmodus cyanostictus (C)	$(TG)_{10}$	315		(AJ407056)
	Eretmodus cyanostictus (C)	$(TG)_{10}$	315		(AJ407055)
	Tanganicodus cf. *irsacae* (D)	$(TG)_9$	313		(AJ407054)
UNH002	*Melanochromis* 'chipokae'	$(CA)_{23}$		52	Kellog *et al*. (1995)
	Tanganicodus irsacae (A)	$(CA)_{13}$	162		(AJ407076)
	Spathodus cf. *erythrodon* (B)	$(CA)_{14}$	164		(AJ407075)
	Eretmodus cyanostictus (C)	$(CA)_{15}$	166		(AJ407077)
TmoM7	*Tropheus moorii*	$(CA)AA(CA)_{10}$		48	Zardoya *et al*. (1996)
	Eretmodus cyanostictus (C)	$(CA)AA(CA)_5$	310		(AJ407059)
	Tanganicodus cf. *irsacae* (E)	$(CA)AA(CA)_6$	312		(AJ407060)
	Tanganicodus cf. *irsacae* (E)	$(CA)AA(CA)_6$	312		(AJ407061)
TmoM13	*Tropheus moorii*	$(CA)_4CG(CG)_{24}$		50	Zardoya *et al*. (1996)
	Spathodus cf. *erythrodon* (B)	$(CA)_8$	201		(AJ407066)
	Eretmodus cyanostictus (C)	$(CA)_7$	199		(AJ407065)
TmoM27†	*Tropheus moorii*	$(CA)_{13}CC(CA)_5$		55	Zardoya *et al*. (1996)
	Eretmodus cyanostictus (C)	$(CA)_5CC(CA)_9$	180		(AJ407050)
	Tanganicodus cf. *irsacae* (D)	$(CA)_5CC(CA)_7$	176		(AJ407051)
UNH001	*Melanochromis* 'chipokae'	$(AG)_5$–19 bp-$(AC)_{35}$		52	Kellog *et al*. (1995)
	Tanganicodus irsacae (A)	$(AG)_5$–19 bp-$(AC)_8$	144		(AJ407074)
	Eretmodus cyanostictus (C)	$(AG)_5$–19 bp-$(AC)_7$	142		(AJ407072)
	Eretmodus cyanostictus (C)	$(AG)_5$–19 bp-$(AC)_7$	142		(AJ407073)
	Tanganicodus cf. *irsacae* (E)	$(AG)_5$–19 bp-$(AC)_7$	142		(AJ407071)

Listed are locus, species for which the locus was originally obtained and eretmodine species for which a specific locus was sequenced (for species designation see Rüber *et al*. 1999), microsatellite core sequence and size of cloned alleles, annealing temperature (T_a), citation for the primer sequences or eretmodine EMBL/GenBank accession numbers (this study), respectively. *For this study a new forward primer was designed: 5′-GCTGTG CTATTTAATCTGTTTATA-3′; †;primers reported in Zardoya *et al*. (1996) did not amplify in eretmodine cichlids and new internal forward 5′-AGCAGTGGAGCAGAGCGAGAACGC-3′ and reverse 5′-AATGTTCTGCGCCACTGGGTCTCC-3′ primers were designed based on the alignment given in Zardoya *et al*. (1996).

sampling interval 1 s). Alleles were sized with the program Allele Links™ (Pharmacia) by using internal standards run in every lane. Internal size standards were obtained by PCR from M13mp18 + template DNA as described in van Oppen *et al*. (1997b). For locus TmoM25, a 453-bp sizer was designed for this study, using the reverse primer 5′-ATTTCGGAACCA CCATCAAA-3′ in combination with the universal M13mp18 + forward primer and an annealing temperature of 54 °C. The following size standards were used: 200 bp + 353 bp; 100 bp + 200 bp; 312 bp + 453 bp; 100 bp + 200 bp; 259 bp + 353 bp; and 100 bp + 200 bp for the loci TmoM5, TmoM11, TmoM25, Pzeb1, Pzeb3, and UNH002, respectively. Allelic corrections were performed for each locus using the sequenced alleles as references (Table 1) and, in addition, for each locus 2–3 randomly chosen individuals were run on all gels to ensure consistency of fragment sizing.

mtDNA

The proline tRNA with a segment of the mitochondrial control region was amplified using conditions and primers described in Rüber *et al*. (1999). Samples were sequenced using the AutoCyle™ Sequencing kit (Pharmacia) and run on an ALF express DNA sequencer (Pharmacia). Some samples were sequenced on an Applied Biosystem 373 A DNA sequencer using the Taq Dye Deoxy Terminator Cycle Sequencing Kit FS (PE Biosystems). The nucleotide sequence data were deposited in EMBL/GenBank under accession numbers AJ407050–AJ407077 for the sequenced microsatellite alleles and AJ407078–AJ407147 for the control region sequences.

Population genetic analyses

Genetic diversity based on microsatellite DNA and mtDNA was assessed for all populations. For the microsatellite data the genetic polymorphism was estimated for each population with GENEPOP 3.1d (Raymond & Rousset 1995) as the number of alleles per locus (N_A), the observed (H_O) and the expected heterozygosity (H_E). Samples were then tested for departures from Hardy–Weinberg equilibrium using probability tests with significance determined by the Markov chain method (Guo & Thompson 1992). Genotypic linkage disequilibrium was evaluated with GENEPOP 3.1d for each pair of loci in each population and significance was determined through a log-likelihood based exact test (Goudet *et al*. 1996). mtDNA polymorphism was assessed by estimating both haplotype (H; Nei 1987) and nucleotide (π; Tajima 1983) diversity within populations. Differences in genetic diversity between the two species were assessed using the Mann–Whitney *U*-test (Sokal & Rohlf 1995).

Population structure

We tested for differences between populations in their allelic (microsatellites) or haplotypic (mtDNA sequences) distributions with an exact test of population differentiation (Raymond & Rousset 1995) as implemented in ARLEQUIN 2.000 (Schneider *et al*. 2000). The significance of the results was determined by the Markov chain method. For the microsatellite data, multilocus probabilities were produced using Fisher's (1954) method of combining probabilities. We then quantified the extent of genetic differentiation among samples by calculating Weir & Cockerham's (1984) estimators of *F*-statistics (θ_{ST}) with ARLEQUIN 2.000. Significance of results was obtained through 5000 permutations. Table-wide rejection levels for multiple tests were calculated with sequential Bonferroni adjusted *P*-values throughout the analyses (Rice 1989).

We performed an analysis of molecular variance (AMOVA), as described in Michalakis & Excoffier (1996), in order to partition the genetic diversity (based either on allele or haplotype frequency) into: (i) variance between species; (ii) variance among populations within species; and (iii) variance within populations. The significance of the variance components associated with the different levels of genetic structure was tested using nonparametric permutation procedures as implemented in ARLEQUIN 2.000.

For the microsatellite data, we assessed relationships among populations using chord distances (D_{CE}; Cavalli-Sforza & Edwards 1967), computed for all pairwise comparisons with PHYLIP version 3.572c (Felsenstein 1995). Pairwise distances were used to build a neighbour-joining (NJ) tree (Saitou & Nei 1987) and robustness of the tree topology was assessed through 1000 bootstrap replications (Felsenstein 1985). Phylogenetic relationships among mtDNA haplotypes were estimated using the NJ method based on Kimura 2-parameter corrected distances (Kimura 1980). Robustness of the inferred NJ tree was tested with PAUP* version 4.0b4a (Swofford 1997) using the bootstrap method with 1000 resamplings.

Finally, we explored the demographic history of the two species. To detect recent effective population size reductions from the microsatellite allele frequencies we used the program BOTTLENECK 1.2.02 (Cornuet & Luikart 1996). This program uses the Sign and Wilcoxon tests to test for an excess of the observed heterozygosity which, for loci evolving under the infinite allele model (IAM), should be larger in populations having experienced a recent bottleneck than the heterozygosity expected for the observed number of alleles in populations under mutation-drift equilibrium. Another bottleneck signature tested with BOTTLENECK 1.2.02 is a deviation of the allele frequency distribution from the approximately L-shaped distribution expected under mutation-drift equilibrium (Luikart *et al*. 1998). To test for a population expansion we applied Fu's (1997) F_s-test of neutrality as implemented in ARLEQUIN 2.000, which is based on the comparison of rare and frequent mutations. After an episode of population growth, the coalescent theory predicts an excess of low frequency alleles and recent mutations in nonrecombining sequences compared to populations at equilibrium (Slatkin & Hudson 1991).

Results

Microsatellite DNA

Of 10 microsatellite loci tested six were chosen for population analyses on the basis of the repeat number in the sequenced alleles (Table 1). The selected loci showed at least 10 uninterrupted dinucleotide repeats in one of the individuals studied and were, therefore, considered to exhibit sufficient potential for polymorphism for population analyses. The six selected loci were: TmoM5, TmoM11, TmoM25, Pzeb1, Pzeb3, and UNH002.

Table 2 Summary of genetic variation detected at six microsatellite DNA loci within seven eretmodine populations from the south-western part of Lake Tanganyika (Democratic Republic of Congo)

	Eretmodus cyanosticus				*Tanganicodus* cf. *irsacae*		
Locus	KAM (30)	KIK (34)	KOR (32)†	KYE (21)	KAM (25)	KAP (26)	KOR (18)
TmoM5 (41)							
N_A	25	24	22	18	14	19	12
H_O	1.00	0.94	0.91	0.90	0.72	1.00	0.89
H_E	0.94	0.95	0.90	0.92	0.85	0.95	0.91
Min (bp)	274	274	272	268	284	286	282
Max (bp)	338	346	344	326	350	348	348
Mode (bp)	274	274	272	268	298	286 + 308	312
TmoM11 (14)							
N_A	6	7	7	3	6	2	3
H_O	0.67	0.53	0.86	0.62	0.28***	0.04	0.17
H_E	0.56	0.52	0.73	0.58	0.64	0.04	0.16
Min (bp)	153	153	147	159	153	167	165
Max (bp)	173	159	169	163	173	169	169
Mode (bp)	159	175	157	159	165	167	167
TmoM25 (22)							
N_A	14	17	14	9	7	8	6
H_O	0.90	0.79	0.72**	0.90	0.76	0.73	0.61
H_E	0.88	0.89	0.93	0.86	0.67	0.69	0.60
Min (bp)	330	348	356	344	348	350	350
Max (bp)	392	386	382	368	378	374	374
Mode (bp)	352	354	364	354	356	350	350
Pzeb1 (15)							
N_A	11	10	2	9	5	3	4
H_O	0.77	0.71	0.25	0.81	0.80	0.50	0.50
H_E	0.82	0.81	0.38	0.79	0.79	0.62	0.53
Min (bp)	124	124	124	132	124	124	124
Max (bp)	146	164	126	156	144	138	140
Mode (bp)	124	124	126	140	138	124	124
Pzeb3 (8)							
N_A	6	8	5	4	1	1	1
H_O	0.50***	0.38***	0.53*	0.48	0.00	0.00	0.00
H_E	0.82	0.73	0.58	0.65	0.00	0.00	0.00
Min (bp)	313	313	311	315	313	313	313
Max (bp)	325	329	321	325	313	313	313
Mode (bp)	321	319	313	325	313	313	313
UNH002 (22)							
N_A	16	12	16	8	1	2	2
H_O	0.90	0.82	0.97	0.57*	0.00	0.15	0.39
H_E	0.91	0.85	0.91	0.67	0.00	0.14	0.32
Min (bp)	154	158	160	162	160	160	160
Max (bp)	200	188	214	188	160	162	162
Mode (bp)	178	168	170	162	160	160	160
TOTAL							
(A)	13.0 ± 7.16	13.0 ± 6.45	11.0 ± 7.59	8.50 ± 5.32	5.70 ± 4.80	5.80 ± 6.91	4.70 ± 3.98
(H_0)	0.79 ± 0.18	0.70 ± 0.21	0.71 ± 0.27	0.71 ± 0.18	0.43 ± 0.38	0.40 ± 0.41	0.43 ± 0.32
(H_E)	0.82 ± 0.14	0.79 ± 0.15	0.74 ± 0.22	0.75 ± 0.13	0.49 ± 0.39	0.41 ± 0.40	0.42 ± 0.33

Sample size is indicated in parentheses beneath each population. The total number of alleles scored for each locus is indicated in parentheses next to the locus name. N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. Allelic diversity (A), average observed (H_O), and expected Hardy–Weinberg (H_E) heterozygosity with standard deviation for each population. Size range of alleles: Min (bp), Max (bp), and Mode (bp) are provided for each population and locus. †Three *E. cyanostictus* individuals from KOR had one allele of size 127 at locus TmoM11, which is 10 bp shorter than the entire microsatellite flanking region, suggesting deletion in the flanking region. For these individuals, genotypes were coded as missing. Significant deviations from Hardy–Weinberg equilibrium: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The core sequences of four of the 10 microsatellite loci sequenced differed from those reported in the original studies. One out of two sequenced eretmodine specimens showed a perfect $(AC)_{33}$ repeat compared to a compound-perfect $(GC)_n(AC)_n$ repeat reported for locus TmoM5 in *Tropheus moorii* (Zardoya *et al*. 1996). In eretmodine cichlids locus TmoM25 had imperfect $(CA)_nCGT(CA)_n$ alleles, compared to perfect $(CA)_n$ alleles found in *T. moorii* (Zardoya *et al*. 1996). The two eretmodine species sequenced for TmoM13 showed a perfect $(CA)_n$ repeat motif compared to a $(CA)_n(CG)_n$ repeat motif found in *T. moorii* (Zardoya *et al*. 1996). The sequences available for locus Pzeb1 showed differences in the repeat in all species examined so far, and further indicate the presence of a poly T region following the microsatellite that also varies in length among alleles (Table 1). In eretmodines the repeat motives $(GT)_nGA(T)_n$ and $(GT)_nTTGC(T)_n$ were found.

Estimates of variability at the six microsatellite DNA loci within populations are shown in Table 2, and allele frequencies are presented in Appendix I. The total number of detected alleles per locus ranged from eight in Pzeb3 to 41 in TmoM5. The mean number of alleles (A) and expected heterozygosity (H_E) across populations are significantly smaller (Mann–Whitney *U*-test; $P = 0.05$) in the *Tanganicodus* populations than in the *Eretmodus* populations (e.g. A, 4.7–5.7 compared to 8.5–13; H_E, 0.41–0.49 compared to 0.74–0.82). At locus Pzeb3 all *Tanganicodus* populations are fixed for allele 313, which only occurs in a high frequency (> 0.500) in *Eretmodus* KOR. At locus UNH002 the *Tanganicodus* populations show either one or two alleles (160 and 162). Allele 160 is absent in the *Eretmodus* populations from KAM and KYE, and allele 162 is absent in *Eretmodus* from KOR (Appendix I).

Six of 42 single-locus tests for deviation from Hardy–Weinberg equilibrium gave significant results, but only three [TmoM11 in *Tanganicodus* (KAM) and Pzeb3 in *Eretmodus* (KAM and KIK)] remained significant when adjusted for table-wide significance by a sequential Bonferroni procedure. Linkage disequilibrium was observed in three out of 105 pairwise comparisons [E. *cyanostictus* KAM, TmoM5 and TmoM25 ($P = 0.050$), TmoM25 and Pzeb1 ($P = 0.015$); *E. cyanostictus* KYE, TmoM25 and Pzeb3 ($P = 0.007$)] but none of them remained significant after sequential Bonferroni correction.

mtDNA

The 198 DNA sequences comprised 70 mitochondrial haplotypes (Table 3 and Appendix II). The overall haplotype and nucleotide diversity were 0.947 and 0.040, respectively. Haplotype diversity was significantly smaller (Mann–Whitney *U*-test; $P = 0.05$) for the *Tanganicodus* populations than for the *Eretmodus* populations but not so for the nucleotide diversity (see Discussion). For these comparisons, two populations (*Eretmodus* KAP and *Tanganicodus* KIK) were left out due to small sample sizes. Two haplotypes (37 and 66) were shared among individuals classified as *Eretmodus* and *Tanganicodus* (Appendix II).

Table 3 Genetic diversity in the mtDNA control region within *Eretmodus* and *Tanganicodus* populations

	N	NH	S	H	π
Eretmodus					
KAM	30	15	18	0.878 ± 0.045	0.0066 ± 0.004
KIK	34	24	26	0.975 ± 0.014	0.0112 ± 0.006
KAP	6	4	4	0.800 ± 0.174	0.0071 ± 0.005
KOR	32	10	9	0.853 ± 0.040	0.0062 ± 0.004
KYE	21	12	10	0.905 ± 0.048	0.0064 ± 0.004
Tanganicodus					
KAM	25	4	18	0.417 ± 0.115	0.0127 ± 0.007
KIK	6	4	24	0.867 ± 0.129	0.0369 ± 0.022
KAP	26	4	7	0.286 ± 0.112	0.0042 ± 0.003
KOR	18	3	7	0.582 ± 0.061	0.0035 ± 0.003
TOTAL	198	70	63	0.947 ± 0.009	0.0404 ± 0.020

Listed are: *N*, number of individuals; NH, number of haplotypes; S, number of segregating sites; H, average haplotype diversity ± SD, average nucleotide diversity ± SD.

Population structure

Across all populations, differentiation tests based on microsatellites showed highly significant heterogeneity of allele frequencies ($P << 0.001$). Single locus pairwise tests were all significant except for eight comparisons [*E. cyanostictus* KAM and KIK (TmoM11, TmoM25, Pzeb1); *E. cyanostictus* KIK and KYE (TmoM25); *T.* cf. *irsacae* KOR and KAP (TmoM5, TmoM25, UNH002); *T.* cf. *irsacae* KAM and KAP (UNH002)]. Multilocus probabilities of heterogeneity between population pairs were all highly significant ($P < 0.001$; for *T.* cf. *irsacae* KOR and KAP, $P < 0.01$).

Based on the microsatellite data, estimates of overall θ_{ST} indicated significant levels of genetic variance among all populations ($\theta_{ST} = 0.250$, $P << 0.001$). For the four *Eretmodus* populations the value was $\theta_{ST} = 0.127$ ($P << 0.001$) and for the three *Tanganicodus* populations it was $\theta_{ST} = 0.240$ ($P << 0.001$). All population pairwise estimates of multilocus θ_{ST} were significantly different from zero (Table 4). Our estimates of fixation indices are not substantially affected and remain significantly different from zero when loci TmoM11 and Pzeb3, which showed significant deviation from Hardy–Weinberg equilibrium in a few populations (see Table 2), are excluded.

Population differentiation tests based on mtDNA haplotype frequencies were highly significant for all pairwise comparisons ($P << 0.001$; *E. cyanostictus* KAM and KIK, $P = 0.0015$). Overall, estimates of θ_{ST} indicated significant

	E. cyanostictus				*T.* cf. *irsacae*		
	KAM	KIK	KOR	KYE	KAM	KAP	KOR
KAM		0.024**	0.138**	0.094**	0.294**	0.338**	0.313**
KIK	**0.037***		0.162**	0.121**	0.299**	0.346**	0.323**
KOR	**0.135****	**0.086****		0.227**	0.266**	0.272**	0.290**
KYE	**0.109****	**0.059****	**0.122****		0.382**	0.365**	0.410**
KAM	**0.344****	**0.289****	**0.354****	**0.348****		0.295**	0.297**
KAP	**0.408****	**0.351****	**0.350****	**0.421****	**0.649****		0.040*
KOR	**0.258****	**0.205****	**0.223****	**0.252****	**0.509****	**0.258****	

Significance of F_{ST}-values: *$P < 0.01$; **$P < 0.001$.

Table 4 Pairwise estimates of overall genetic divergence between populations of *Eretmodus cyanostictus* and *Tanganicodus* cf. *irsacae* obtained using six microsatellite loci (above diagonal, plain text) and mtDNA control regions (below diagonal, in bold). F_{ST} estimated with ARLEQUIN 2.000 (Schneider *et al.* 2000) as theta (Weir & Cockerham 1984)

levels of genetic variance among all populations ($\theta_{ST} = 0.263$, $P << 0.001$). For the four *Eretmodus* populations the value was $\theta_{ST} = 0.089$ ($P << 0.001$) and for the three *Tanganicodus* populations it was $\theta_{ST} = 0.526$ ($P << 0.001$). Population pairwise estimates of θ_{ST} were all significantly different from zero (Table 4).

The AMOVA revealed that 18.4% of the total variance in microsatellite allele frequency was due to between species heterogeneity ($F_{CT} = 0.184$) and 12.9% was caused by differences among populations within species ($F_{SC} = 0.158$). The remainder (68.7%) was due to variation within populations ($F_{ST} = 0.312$). For the mtDNA data, the corresponding values were 7.8% ($F_{CT} = 0.078$), 21.1% ($F_{SC} = 0.228$) and 71.1% ($F_{ST} = 0.289$, Table 5).

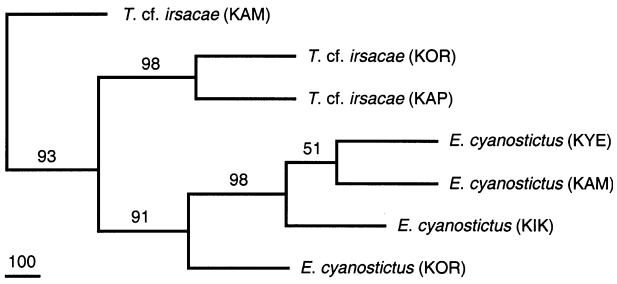


Fig. 2 Unrooted, majority-rule consensus tree representing 1000 bootstrap replicates of chord distance data (D_{CE}), cluster with the neighbour-joining algorithm. Bootstrap values based on 1000 replications are shown above branches.

Population clustering and phylogenetic analysis

A majority-rule consensus NJ tree (based on 1000 bootstrap-replicated chord distance matrices) grouped most populations by species rather than geographical location (Fig. 2). All except one internal node were well supported (i.e. bootstrap values > 70%). For example, the node grouping all *Eretmodus* was supported by a bootstrap value of 91% (Fig. 2).

The NJ phylogeny of the 70 identified mtDNA haplotypes shows a basal split into two genetically distinct lineages (bootstrap values 94% and 86%; Fig. 1). These lineages were previously defined as the *E. cyanostictus* lineage C and the *T.* cf. *irsacae* lineage D (Rüber *et al.* 1999). Within lineage C two major clades were observed, one comprising all the *E. cyanostictus* from KYE (clade C-1, bootstrap value 100%) and another that includes all individuals of *E. cyanostictus* from the localities KAM and KIK (clade C-2, bootstrap value 75%). Clade C-2 also includes a haplotype shared by one individual of *E. cyanostictus* from KIK and one of *T.* cf. *irsacae* from KIK (37), and three haplotypes found in a few individuals of *T.* cf. *irsacae* from either KAM or KIK (38–40, Appendix II).

Within lineage D three major clades were found (bootstrap values 62%, 68% and 97%). Clade D-3 comprises four haplotypes (53–56) representing 24 individuals of *T.* cf. *irsacae* from KAM and KIK. Clade D-2 containes haplotypes 68 and 70 from three *T.* cf. *irsacae* (localities KAP and KOR). And finally, clade D-1 contains 12 haplotypes. In this clade, haplotypes 57–65 are found in *E. cyanostictus* from KAP and KOR; haplotype 66 is found in four *E. cyanostictus* from KOR, in nine *T.* cf. *irsacae* from KOR, and in 22 *T.* cf. *irsacae* from KAP; the remaining two haplotypes (67 and 69) are found in eight *T.* cf. *irsacae* from KOR and in one *T.* cf. *irsacae* from KAP. The average sequence divergence between individuals from lineage C and D was 6.20% ± 0.12% (net sequence divergence corrected for intrapopulation polymorphism was 4.45% ± 0.15%). Within lineage C and D the average sequence divergence between individuals was 2.56% ± 0.10 and 1.36% ± 0.03, respectively. Applying a molecular clock calibration based on cichlid control region sequences (5.6% per site per 10^6 years; Nagl *et al.* 2000) on the net sequence divergence suggests that the two lineages (C and D) separated roughly 0.8 million years ago.

Demographic history

Both the Sign and the Wilcoxon tests gave no indication for heterozygote excess, and the allele frequency distributions indicated no deviation from an approximate L-shaped distribution in any of the populations. Thus, these results indicate that, according to the microsatellite data, none of the observed populations underwent a recent bottleneck. Fu's test supported a demographic expansion of the *Eretmodus* populations with the exception of *Eretmodus* KOR (F_S-values ranged from −6.91 to −13.26; $P \leq 0.0001$; *Eretmodus* KOR $F_S = -2.79$, $P = 0.0718$. All *Tanganicodus* populations showed positive F_S-values and are therefore compatible with population stasis (F_S-values ranged from 1.16 to 5.54; $P > 0.750$).

Table 5 Hierarchical analysis of molecular variance based on six microsatellite DNA loci and mtDNA control region sequences in *Eretmodus cyanostictus* and *Tanganicodus* cf. *irsacae* populations

Variance component Source of variation	V	%	Fixation indices		*P*
Microsatellites					
Between species	0.52	18.37	F_{CT}	0.184	< 0.0000
Among populations/ within species	0.37	12.88	F_{SC}	0.158	< 0.0000
Within populations	1.96	68.75	F_{ST}	0.312	< 0.0000
mtDNA sequences					
Between species	0.04	7.80	F_{CT}	0.078	< 0.0000
Among populations/ within species	0.11	21.09	F_{SC}	0.228	< 0.0000
Within populations	0.36	71.11	F_{ST}	0.289	0.0508

Discussion

Microsatellite sequences

In eretmodine cichlids four out of 10 loci have undergone mutational events in the microsatellite repeats, compared to the species from which the microsatellites were obtained (Table 1). Differences in the repeat motif as found here underscore the need for more DNA sequence data to adequately assess genetic variability within and among species, and to understand the evolutionary processes of these markers (e.g. Zardoya *et al*. 1996). Such information may also prove essential to correct for size-homoplasies in estimating demographic population parameters such as structure, gene flow, and effective population size (e.g. Angers & Bernatchez 1997). Another illustration of this issue was given in a recent study that revealed extensive size homoplasy at locus Pzeb4 within and among 11 Lake Malawi cichlids species (van Oppen *et al*. 2000). These authors further cautioned against using population parameters based on the stepwise mutation model (SMM) if conformance to SMM for the loci used were not demonstrated. Our results indicate that loci TmoM11, Pzeb3, and UNH002, which exhibit perfect dinucleotide repeats in all the cichlid species studied so far, might be less affected by size homoplasy than other loci that show more complex mutations at the intra- and interspecific level (Table 1). Further comparative data of other cichlid species might help to distinguish loci that are more affected by size homoplasy than others.

Intra- and interspecific genetic variation

Genetic variation inferred from mtDNA sequences and microsatellites was consistently lower in the *Tanganicodus* than in the *Eretmodus* populations (Tables 2 and 3). Only the nucleotide diversity did not differ significantly between the two species due to haplotypes 39 and 40 found in *T*. cf. *irsacae* at KAM (Table 3) that clustered with *Eretmodus* haplotypes in clade C-2 (Fig. 1). Excluding these haplotypes from the analysis reduces the nucleotide diversity of the *Tanganicodus* KAM population to 0.0005 ± 0.001 resulting in a significantly lower nucleotide diversity in all the *Tanganicodus* populations in comparison to the *Eretmodus* populations (Mann–Whitney *U*-test; $P = 0.05$). The relatively high nucleotide diversity observed in *Tanganicodus* KIK, which was not included in the analyses (see Results), can also be explained by the presence of two haplotypes (37 and 38, found in three individuals) that clustered with clade C-2 (Fig. 1).

The observed species-specific differences in genetic diversity suggest that the two species might have had different evolutionary histories in terms of random genetic drift, variation of effective population size, or natural selection. We estimated N_E for the *Eretmodus* and *Tanganicodus* populations using the formula $\pi = 4N_E\mu$, where π is the nucleotide diversity and μ is the mutation rate assumed to be 2.8×10^{-8} (substitutions × base pair^{-1} × year^{-1}) for the control region in cichlids (Nagl *et al*. 2000). For the *Eretmodus* populations the estimated N_E is in the order of 2–4×10^5, whereas for the *Tanganicodus* populations it is in the order of 2–10×10^4. Parker & Kornfield (1997) and Nagl *et al*. (1998) obtained estimates of either N_f or N_E of similar magnitude for several species of lakes Malawi and Victoria cichlids. The difference in the estimated effective population size between the two eretmodine species agrees with field observations by Hori *et al*. (1983), who studied the abundance and microdistribution of cichlids on a rocky shore in the northern part of the lake and recorded higher abundance of *E*. cf. *cyanostictus* (lineage A) than of *T. irsacae* (lineage A).

The mtDNA control region sequences revealed a relatively large amount of polymorphism in the *Eretmodus* populations in comparison to other studies on lacustrine cichlids. For example, Meyer *et al*. (1996) found an overall

nucleotide diversity in the mtDNA control region of 0.0026 and 0.0037 in two *Simochromis* species (25 and 28 individuals studied) from Lake Tanganyika collected over a shoreline of 440 km. Bowers *et al*. (1994) found an overall nucleotide diversity of 0.0008 and 0.0050 in two species of the mbuna genus *Melanochromis* from the southern part of Lake Malawi (68 and 42 individuals studied). They also found that 75% of the populations were fixed for a single haplotype. A collection of all available Lake Malawi *mbuna* control region sequences revealed 58 control region haplotypes with a total of 46 segregating sites among 180 individuals from 34 species representing several *mbuna* genera [Parker & Kornfield (1997); see Table 3 for a comparison with the two species studied in this paper]. The low levels of mtDNA variation found in many Lake Malawi *mbunas* may be due to recent colonization of habitat patches from refugia populations in the recent past due to lake level fluctuations (van Oppen *et al*. 1997a; Arnegard *et al*. 1999; Markert *et al*. 1999; Sturmbauer *et al*. 2001). The level of expected heterozygosity based on microsatellite data found in the *Eretmodus* populations (0.72 average across all loci and all populations), is comparable with data for *Melanochromis auratus* (0.67; Markert *et al*. 1999), *Labeotropheus fulleborni* (0.83; Arnegard *et al*. 1999) and for four *Pseudotropheus* species (0.72 average over species; van Oppen *et al*. 1997a) from Lake Malawi. In the light of the findings from this and other studies, *T*. cf. *irsacae* is the genetically least polymorphic lacustrine cichlid species found so far. There is no evidence for a recent population bottleneck that may account for the lower genetic variation found in the *Tanganicodus* populations. On the other hand, we found evidence of a population expansion in *Eretmodus*, the species that shows higher genetic diversity. With the information at hand it is not possible to determine whether the higher genetic diversity in *Eretmodus* is only associated with the hypothesized expansion or if there are additional species-specific differences in life history traits and/or environmental factors that might affect their population sizes.

Population structure

Both microsatellite markers and mtDNA sequences revealed significant levels of genetic structure within the two sympatric eretmodine species — along 138 km of coastline for the *Eretmodus* populations and over a distance of 35 km for the *Tanganicodus* populations. The higher degree of population structuring observed in *Tanganicodus* may be attributed to the lower levels of genetic diversity, in both microsatellites and mtDNA markers within populations, that influence measures of population differentiation (e.g. Nagylaki 1998). Other factors such as dispersal ability and phylopatry may contribute to differences in population structure between the two species.

Among the 70 identified haplotypes, shared haplotypes were never found between more than two neighbouring populations. For example the *Eretmodus* populations at KAM and KIK with 15 and 24 haplotypes, respectively, separated by a distance of 7 km of rocky shore, shared only two haplotypes. In the *Tanganicodus* populations (Appendix II) haplotype 67 was observed in over 40% of the individuals from KOR, but not at KAP which is situated only 7 km away. Such a low frequency of shared mtDNA haplotypes among localities is likely to be an indicator for the expressed site fidelity of eretmodine cichlids, which may rapidly lead to genetic isolation among populations. Although the amount of shared mtDNA haplotypes may change with increasing sample size, our microsatellite results support a high degree of genetic isolation between populations.

Philopatry coupled with the patchy distribution of rocky habitats along the lake shores have been identified as important factors for population divergence among Lake Malawi *mbuna* cichlids (van Oppen *et al*. 1997a; Arnegard *et al*. 1999; Markert *et al*. 1999). The availability of suitable habitat patches as well as their distribution is strongly affected by lake level fluctuations. A recent study confirmed that lake level fluctuations in Lake Tanganyika, induced by tectonic and/or climatic events, have strongly affected the distribution and fragmentation of rocky habitat patches in the littoral (Cohen *et al*. 1997). Cohen *et al*. (1997) identified seven lake lowstand periods in the Northern Tanganyika basin that have been named and dated as follows: (f) 1.1 Ma; (d) 393–363 Ka (1 Ka = 1000 years); (c) 295–262 Ka; (b) 193–169 Ka; (a) 40–35 Ka; (x) 23 Ka; (y) 18 Ka. The rise of the lake to its present level was completed 12 Ka (A. Cohen, personal communication; see also Sturmbauer *et al*. 2001). The magnitudes of the lake lowstands below present lake level were given by these authors as 650–700 m for (f); 350 m for (d); 350 m for (c), 250 m for (b) and 160 m for (a). Gasse *et al*. (1989) give estimates of 300–400 m for the magnitude of the late Pleistocene lowstands (x) and (y). Other authors suggest them to be more likely in the order of 150–200 m (A. Cohen, personal communication). Lake level fluctuations in the Holocene were less severe in Lake Tanganyika (maximum 75 m; see Coulter 1991) compared to Lake Malawi where it has been reported that a lake level drop of 120 m occurred 200 years ago (Owen *et al*. 1990).

As they may cause fusion or isolation of populations, water level changes in shallow areas of the lake are likely to have severe effects on population structure. At steeply sloping shores, lake level fluctuations will mostly cause a vertical dislocation of the populations and, as in these zones rocky habitats often extend far into deep water, are less likely to promote cycles of fusions and isolations of populations (Sturmbauer *et al*. 1997; Sturmbauer 1998). This is the case for all localities analysed in this study. We

provide evidence for significant population differentiation along a mostly continuous rocky shoreline that is only rarely interrupted by few small sandy bays and influent rivers, with areas of sand deposition that might act as barriers to gene flow, and that may not have been much affected by lake level fluctuations. A question that needs to be studied in more detail is whether the small sandy beaches observed in the study area act as effective dispersal barriers and significantly contribute to population differentiation in the rock-dwelling eretmodine cichlids, or whether divergence also occurs under parapatric conditions —, i.e. along a continuous habitat (Taylor *et al.* 2001).

Evidence for introgression

Throughout its narrow distribution range *T.* cf. *irsacae* occurs in sympatry with *E. cyanostictus*. Chord distances grouped the *Eretmodus* populations in a separate cluster (Fig. 2). The mitochondrial gene phylogeny (Fig. 1), on the other hand, shows that some individuals of *T.* cf. *irsacae* from KAM and KIK were placed within the *E. cyanostictus* lineage (clade C-2), whereas all individuals of *E. cyanostictus* from KAP and KOR were resolved within the *T.* cf. *irsacae* lineage (clade D-1).

The observed distribution of mitochondrial haplotypes in these two closely related species is incongruent with the morphology-based species assignment which clearly indicates their morphological distinctness. Shared ancient polymorphism as a result of incomplete lineage sorting or introgression during secondary contact may be responsible for this incongruence. At the present state of knowledge we favour the introgression scenario over incomplete lineage sorting. An argument against incomplete lineage sorting is the local occurrence of the mtDNA haplotypes found in lineages C and D that conflict with the species tree. In addition, given the average sequence divergence of 6.20% separating the two lineages, we consider it unlikely that retained ancestral haplotypes in *Eretmodus* at KOR and KAP would be identical or nearly identical to the *Tanganicodus* haplotypes at those localities. There are five fixed substitutions between lineage C and D haplotypes. Lineage D haplotypes show the following diagnostic substitutions: 40T, 123 T, 125 C, 248 A, and 294 C. Two of these sites show transversions (125 A/C and 248 T/A) which are diagnostic for lineage D haplotypes in regard to all other eretmodine lineages [based on a total of 288 eretmodine control region sequences; this study and Rüber *et al.* (1999)]. Therefore, we consider it more likely that the unique transversional mutations at sites 125 and 248 occurred along the branch grouping all lineage D haplotypes rather than it being present as a polymorphism in the shared ancestral gene pool from which *E. cyanostictus*, *T.* cf. *irsacae* and other eretmodine cichlids may have originated.

The possible mtDNA introgression cases we observe in our data are spatially restricted and asymmetric: complete introgression from *Tanganicodus* to *Eretmodus* at KOR and KAP, and partial introgression from *Eretmodus* to *Tanganicodus* at KIK and KAM. In contrast, the microsatellite allele frequency distributions indicate a limited degree of nuclear introgression, although allele 313 at locus Pzeb3 which is fixed in the *Tanganicodus* populations appears in a relatively high frequency in *Eretmodus* at KOR (see Results and Appendix I). Moreover, introgressed populations do not differ phenotypically from nonintrogressed ones. These observations, together with the genetical differentiation observed between sympatric *Tanganicodus* and *Eretmodus* populations (Table 4), suggests that hybridization might have taken place locally for a short period of time during secondary contact, and that there is no ongoing gene flow. Reinforcement may have contributed to the rapid evolution of reproductive isolation of the two sister species upon secondary contact, despite continuing sympatry (Butlin 1989).

The fixation of *Tanganicodus* mtDNA in the introgressed *Eretmodus* populations at KAP and KOR may have occurred either by chance (via founder effects or drift) or by selection. Although we have no indication that selection is responsible for the fixation of *Tanganicodus* mtDNA in the two introgressed *Eretmodus* populations, selective advantages of heterospecific mtDNA have been suggested as explanations of similar cases among salmonid species (Wilson & Bernatchez 1998 and references therein). The proposed direction of mtDNA introgression from *Tanganicodus* to *Eretmodus* at KAP and KOR seems at odds with the observation of higher genetic diversity and hence larger population sizes found in the latter populations. It is possible, however, that small *Eretmodus* founder populations colonized this area of the lake and established secondary contact with *Tanganicodus* populations, and that the presumed expansion of population size in *Eretmodus* happened after the interspecific replacement of mtDNA due to introgression.

There is increasing evidence, particularly from fishes, that mtDNA introgression between allopatrically diverged taxa in zones of secondary intergradation may be more common than previously thought (e.g. Dowling & deMarais 1993; Bernatchez *et al*. 1995; Wilson & Bernatchez 1998). However, it remains to be tested to what extent historic events of introgressive hybridization can explain patterns of shared haplotypes among species, or even genera, in other cichlid species flocks. Incomplete lineage sorting has been postulated for the lakes Malawi and Victoria cichlid flocks (Moran & Kornfield 1993, 1995; Parker & Kornfield 1997; Nagl *et al*. 1998, 2000). As in Lake Tanganyika, intermediate phenotypes are rare or absent in these lakes, suggesting that little or no hybridization is going on at the present time, possibly due to strong assortative mating

(Seehausen *et al*. 1997; Knight *et al*. 1998; van Oppen *et al*. 1998). In contrast to Lake Tanganyika, the ages of these radiations are much younger, which makes incomplete lineage sorting more likely. Nevertheless, introgression caused by, for example, changes in water transparency, which may have occasionally broken down reproductive barriers among multiple species (see Seehausen *et al*. 1997), may in some cases be an alternative hypothesis.

Acknowledgements

We are grateful to Heinz H. Büscher for inviting L.R. to a collection trip to the Democratic Republic of Congo in 1996. Thanks to Rafael Zardoya for his help with the cloning of some of the microsatellites; Paul Shaw and Peter Gallbusera for primer exchange and sharing protocols. L.R. also thanks Sanja Baric, Harald Niederstätter and Walter Salzburger for their help and Hospitality at the Zoological Institute, University of Innsbruck, where parts of the microsatellite cloning and mtDNA sequencing was conducted. We gratefully acknowledge the help of T. Backeljau, K. Martens and S. Villalba for reading and commenting on early drafts of the manuscript and/or discussions. We also thank three anonymous reviewers for useful suggestions and comments on an earlier version of this manuscript. D. Swofford kindly granted permission to publish results based on the test version of PAUP* 4.0d65. This research was supported by grants from the Swiss National Science Foundation (TMR 83EU-045301), and the Gottfried R. Friedli-Stiftung (Bülach, Switzerland) to L.R., the Roche Research Foundation (Basel, Switzerland) to L.R. and A.M. Additional support came from a DWTC project to E.V., US NSF grants BSR-9107838 and DEB-9615178 and support from the University of Konstanz to A.M and grant P12339-GEN from the Austrian research Foundation to C.S.

References

Angers B, Bernatchez L (1997) Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. *Molecular Biology and Evolution*, **14**, 230–238.

Arnegard ME, Markert JA, Danley PD *et al.* (1999) Population structure and colour variation of the cichlid fish *Labeotropheus fuelleborni* Ahl along a recently formed archipelago of rocky habitat patches in southern Lake Malawi. *Proceedings of the Royal Society London, Series B*, **266**, 119–130.

Bernatchez L, Glémet H, Wilson CC, Danzmann RG (1995) Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in a allopatric population of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 179–185.

Bowers N, Stauffer JR, Kocher TD (1994) Intra- and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malawi, Africa. *Molecular Phylogenetics and Evolution*, **3**, 75–82.

Brooks JL (1950) Speciation in ancient lakes. *Quarterly Review of Biology*, **25** (30–36), 131–176.

Butlin R (1989) Reinforcement of premating isolation. In: *Speciation and its Consequences* (eds Otte D, Endler JA), pp. 158–179. Sinauer, Sunderland MA.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution*, **32**, 550–570.

Cohen AS, Lezzar KE, Tiercelin JJ, Soreghan MJ (1997) New palaeogeographic and lake-level reconstructions of Lake Tanganyika: implications for tectonic, climatic and biological evolution in a rift lake. *Basin Research*, **9**, 107–132.

Cohen AS, Soreghan MJ, Scholz CA (1993) Estimating the age of formation of lakes: an example from Lake Tanganyika, East African Rift system. *Geology*, **21**, 511–514.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Coulter GW (1991) *Lake Tanganyika and its Life*. Oxford University Press, Oxford, UK.

Dowling TE, de Marais BD (1993) Evolutionary significance of introgressive hybridization in fishes. *Nature*, **362**, 444–446.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Felsenstein J (1995) PHYLIP — *Phylogeny Inference Package, Version 3.572c*. University of Washington, Seattle.

Fisher RA (1954) *Statistical Methods for Research Workers*. 12th edn. Oliver & Boyd, Edinburgh, UK.

Fryer G, Illes TD (1972) *The Cichlid Fishes of the Great Lakes of Africa: their Biology and Evolution*. Oliver & Boyd, Edinburgh, UK.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Galis F, Metz JAJ (1998) Why are there so many cichlid species? *Trends in Ecology and Evolution*, **13**, 1–2.

Gasse F, Lédée V, Massault M, Fontes JC (1989) Water level fluctuations of lake Tanganyika in phase with oceanic changes during the last glaciation and deglaciation. *Nature*, **342**, 57–59.

Goudet J, Raymond M, de Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Guo SW, Thompson EA (1992) Performing the exact test for Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Hori M, Yamaoka K, Takamura K (1983) Abundance and microdistribution of cichlid fishes on a rocky shore of Lake Tanganyika. *African Study Monographs*, **3**, 25–38.

Huysseune A, Rüber L, Verheyen E (1999) A single tooth replacement pattern generates diversity in the dentition in cichlids of the tribe Eretmodini, endemic to Lake Tanganyika (Teleostei: Cichlidae). *Belgian Journal of Zoology*, **129**, 157–174.

Kellogg KA, Markert JA, Stauffer JR, Kocher TD (1995) Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proceedings of the Royal Society of London, Series B*, **260**, 79–84.

Kimura M (1980) A simple method for estimating the evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Knight ME, Turner GF, Rico C, van Oppen MJH, Hewitt GM (1998) Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. *Molecular Ecology*, **7**, 1605–1610.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science of the USA*, **86**, 6196–6200.

Kuwamura T, Nagoshi M, Sato T (1989) Femal-tomale shift of mouthbrooding in a cichlidn fish, *Tanganicodus irsacae*, with notes on breeding habits of two related species in Lake Tanganyika. *Environmental Biology of Fishes*, **24**, 187–198.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.

Markert JA, Arnegard ME, Danley PD, Kocher TD (1999) Biogeography and population genetics of the Lake Malawi cichlid *Melanochromis auratus*: habitat transience, philopatry and speciation. *Molecular Ecology*, **8**, 1013–1026.

Meyer A (1993) Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology and Evolution*, **8**, 279–284.

Meyer A, Knowles L, Verheyen E (1996) Widespread distribution of mitochondrial haplotypes in rock-dwelling cichlid fishes from Lake Tanganyika. *Molecular Ecology*, **5**, 341–350.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special references to microsatellite loci. *Genetics*, **142**, 1061–1064.

Moran P, Kornfield I (1993) Retention of ancestral polymorphism in the mbuna species flocks (Pisces: Cichlidae) of Lake Malawi. *Molecular Biology and Evolution*, **10**, 1015–1029.

Moran P, Kornfield I (1995) Were population bottlenecks associated with radiation of the *mbuna* species flock (Teleostei: Cichlidae) of Lake Malawi? *Molecular Biology and Evolution*, **12**, 1085–1093.

Nagl S, Tichy H, Mayer WE, Takahata N, Klein J (1998) Persistence of neutral polymorphisms in Lake Victoria cichlids. *Proceedings of the National Academy of Sciences of the USA*, **95**, 14238–14243.

Nagl S, Tichy H, Mayer WE, Takezaki N, Takahata N, Klein J (2000) The origin and age of haplochromine fishes in Lake Victoria, East Africa. *Proceedings of the Royal Society London. Series B*, **267**, 1049–1061.

Nagylaki T (1998) Fixation indices in subdivided populations. *Genetics*, **148**, 1325–1332.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY.

Nishida M (1991) Lake Tanganyika as an evolutionary reservoir of old lineages of East African cichlid fishes: inferences from allozyme data. *Experientia*, **47**, 974–979.

van Oppen MJH, Rico C, Deutsch JC, Turner GF, Hewitt GM (1997b) Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology*, **6**, 387–388.

van Oppen MJH, Rico C, Turner GF, Hewitt GM (2000) Extensive homoplasy, nonstepwise mutations, and shared ancestral polymorphism at a complex microsatellite locus in Lake Malawi cichlids. *Molecular Biology and Evolution*, **17**, 489–498.

van Oppen MJH, Turner GF, Rico C *et al.* (1997a) Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proceedings of the Royal Society London, Series B*, **264**, 1803–1812.

van Oppen MJH, Turner GF, Rico C *et al.* (1998) Assortative mating among rock-dwelling cichlid fishes supports high estimates of species richness from Lake Malawi. *Molecular Ecology*, **7**, 991–1001.

Owen RB, Crossley R, Johnson TC *et al.* (1990) Major low levels of Lake Malawi and their implications for speciation rates in cichlid fishes. *Proceedings of the Royal Society London, Series B*, **240**, 519–553.

Parker A, Kornfield I (1997) Evolution of the mitochondrial DNA control region in the *mbuna* (Cichlidae) species flock of Lake Malawi, East Africa. *Journal of Molecular Evolution*, **45**, 70–83.

Poll M (1952) Ségrégation géographic et formation des espèces. *Annales de la Société Royale Zoologique de Belgique*, **83**, 211–224.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ribbink AJ, Marsh AC, Marsh BA, Sharp BJ (1983) A preliminary survey of the cichlid fishes of the rocky habitats in Lake Malawi. *South African Journal of Zoology*, **18**, 149–310.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rüber L, Adams DC (2001) Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. *Journal of Evolutionary Biology*, **14**, in press.

Rüber L, Verheyen E, Meyer A (1999) Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proceedings of the National Academy of Sciences of the USA*, **96**, 10230–10235.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for constructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Schliewen U, Tautz D, Pääbo S (1994) Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature*, **368**, 629–632.

Schneider S, Rössli D, Excoffier L (2000) ARLEQUIN, *Version 2.000: a Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seehausen O, van Alphen JJM, Witte F (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277**, 1808–1811.

Seehausen O, van Alphen JJM, Witte F (1999) Can ancient colour polymorphisms explain why some cichlid lineages speciate rapidly under disruptive sexual selection? *Belgian Journal of Zoology*, **129**, 43–60.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Snoeks J, Rüber L, Verheyen E (1994) The Tanganyika problem: taxonomy and distribution patterns of its ichthyofauna. *Advances in Limnology*, **44**, 357–374.

Sokal RR, Rohlf FJ (1995) *Biometry*. Freeman, New York.

Sturmbauer C (1998) Explosive speciation in cichlid fishes of the African Great Lakes: a dynamic model of adaptive radiation. *Journal of Fish Biology*, **53** (Suppl. A), 18–36.

Sturmbauer C, Baric S, Salzburger W, Rüber L, Verheyen E (2001) Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. *Molecular Biology and Evolution*, **18**, 144–154.

Sturmbauer C, Meyer A (1992) Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature*, **359**, 578–581.

Sturmbauer C, Meyer A (1993) Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes from Lake Tanganyika in Eastern Africa. *Molecular Biology and Evolution*, **10**, 751–768.

Sturmbauer C, Verheyen E, Rüber L, Meyer A (1997) Phylogeographic patterns in populations of cichlid fishes from rocky habitats in Lake Tanganyika. In: *Molecular Systematics of Fishes* (eds Kocher TD, Stepien CA), pp. 93–107. Academic Press, New York, NY.

Swofford DL (1997) PAUP*: *Phylogenetic Analysis Using Parsimony* (*and Other Methods), *Version 4.0*. Sinauer Associates, Sunderland, MA.

Tajima F (1983) Evolutionary relationships of DNA sequences in finite populations. *Genetics*, **105**, 437–460.

Taylor MI, Rüber L, Verheyen E (2001) Biparental mouthbrooding and monogamy are correlated with high levels of population substructuring in *Eretmodus cyanostictus*, a rock-dwelling cichlid from Lake Tanganyika. *Proceedings of the Royal Society London, Series B*, **268**, in press.

Weir B, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wilson CC, Bernatchez L (1998) The ghost of hybrids past: fixation of arctic charr (*Savelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). *Molecular Ecology*, **7**, 127–132.

Yamaoka K, Hori M, Kuratani S (1986) Ecomorphology of feeding in 'goby-like' cichlid fishes in Lake Tanganyika. *Physiology and Ecology Japan*, **23**, 17–29.

Zardoya R, Vollmer D, Craddock C, Streelman J, Karl S, Meyer A (1996) Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proceedings of the Royal Society London, Series B*, **263**, 1589–1598.

This study was part of Lukas Rüber's PhD research applying molecular genetic techniques and morphometric analyses to study the evolutionary history of eretmodine cichlids from Lake Tanganyika. Axel Meyer is Professor in the Department of Biology at the University of Konstanz and interested in molecular phylogenetics and evolutionary biology. Christian Sturmbauer is Associate Professor at the Department of Zoology and Limnology at the University of Innsbruck and interested in molecular systematics and evolutionary processes in cichlids and a variety of other organisms. Erik Verheyen is a staff scientist at the Royal Belgian Institute of Natural Sciences in Brussels and is working on several projects involving the role of palaeoclimatic changes on speciation and evolution of selected African vertebrates. L.R is postdoctoral fellow in the laboratory of Rafael Zardoya at the Museo Nacional de Ciencias Naturales in Madrid.

Appendix I

Allele frequencies at six microsatellite loci for the *Eretmodus* and *Tanganicodus* populations

	Eretmodus cyanostictus				*Tanganicodus* cf. *irsacae*		
Allele length (bp)	KAM	KIK	KOR	KYE	KAM	KAP	KOR
TmoM5							
268	—	—	—	0.190	—	—	—
272	—	—	0.281	—	—	—	—
274	0.200	0.162	0.109	0.048	—	—	—
276	0.033	0.074	0.063	—	—	—	—
278	—	—	0.016	—	—	—	—
280	0.017	—	—	—	—	—	—
282	0.017	—	—	—	—	—	0.028
284	—	0.088	—	—	0.240	—	—
286	0.067	—	—	—	0.020	0.135	0.111
288	0.050	—	—	—	—	—	—
290	—	—	—	0.024	—	—	—
292	0.033	0.015	0.031	0.048	—	—	0.056
294	0.033	0.044	0.031	—	0.040	0.038	—
296	0.100	0.044	0.016	0.024	0.120	—	—
298	0.017	0.015	—	—	0.280	—	—
300	—	0.059	0.078	0.024	0.040	0.019	—
302	—	0.074	0.031	0.048	—	0.058	—
304	0.033	0.015	0.031	0.024	—	—	0.028
306	0.017	0.029	0.031	0.048	0.060	0.058	—
308	0.067	0.059	0.031	0.024	0.020	0.135	0.111
310	0.050	0.015	—	0.024	—	0.058	0.111
312	0.033	—	0.016	0.071	—	0.077	0.194
314	0.033	0.059	0.016	0.143	—	0.038	0.111
316	—	0.015	—	0.143	—	0.058	0.139
318	0.017	0.044	0.047	0.024	—	0.038	0.028
320	0.050	—	—	0.024	—	0.019	—
322	—	—	0.078	0.024	—	0.038	—
324	0.017	0.044	—	—	0.020	—	—
326	0.017	0.029	0.016	0.048	—	—	—
328	0.017	0.029	0.016	—	—	—	—
330	0.033	—	0.016	—	—	—	—
332	0.017	—	—	—	0.020	0.058	0.056
334	0.017	0.015	0.016	—	0.020	0.058	—
336	—	0.015	0.016	—	—	0.038	—
338	0.017	0.015	—	—	0.040	0.038	—
340	—	—	—	—	—	0.019	—
342	—	0.015	—	—	—	—	—
344	—	—	0.016	—	—	—	—
346	—	0.029	—	—	—	—	—
348	—	—	—	—	0.020	0.019	0.028
350	—	—	—	—	0.060	—	—
N	60	68	64	42	50	52	36
TmoM11							
147	—	—	0.155	—	—	—	—
149	—	—	—	—	—	—	—
151	—	—	—	—	—	—	—
153	0.200	0.118	0.034	—	0.020	—	—
155	0.083	0.118	—	—	0.040	—	—
157	—	—	0.431	—	—	—	—
159	0.633	0.676	—	0.476	0.060	—	—
161	0.017	—	0.259	0.452	—	—	—

Appendix I *continued*

	Eretmodus cyanostictus				*Tanganicodus* cf. *irsacae*		
Allele length (bp)	KAM	KIK	KOR	KYE	KAM	KAP	KOR
163	0.017	—	0.017	0.071	—	—	—
165	—	0.029	0.017	—	0.560	—	0.056
167	—	—	—	—	—	0.981	0.917
169	—	—	0.086	—	0.100	0.019	0.028
171	—	0.015	—	—	—	—	—
173	0.050	0.029	—	—	0.220	—	—
175	—	0.015	—	—	—	—	—
N	60	68	58	42	50	52	36
TmoM25							
330	0.017	—	—	—	—	—	—
344	—	—	—	0.048	—	—	—
348	—	0.015	—	—	0.060	—	—
350	0.017	0.015	—	0.143	—	0.500	0.611
352	0.217	0.132	—	0.167	—	0.019	—
354	0.033	0.235	—	0.262	0.140	0.231	0.083
356	0.067	0.059	0.063	0.167	0.540	—	—
358	0.200	0.118	0.047	0.095	—	0.077	—
360	0.117	0.074	0.031	0.024	0.140	0.077	—
362	0.067	0.132	0.063	0.048	0.040	—	0.028
364	0.083	0.044	0.156	—	0.040	0.019	0.028
366	0.033	0.029	0.094	—	—	—	—
368	—	0.015	0.094	0.048	—	0.038	0.139
370	0.100	0.044	0.094	—	—	—	—
372	0.017	0.029	0.094	—	—	—	—
374	—	0.015	0.078	—	—	0.038	0.111
376	—	—	0.047	—	—	—	—
378	0.017	0.015	0.063	—	0.040	—	—
380	—	0.015	0.063	—	—	—	—
382	—	—	0.016	—	—	—	—
386	—	0.015	—	—	—	—	—
392	0.017	—	—	—	—	—	—
N	60	68	64	42	50	52	36
Pzeb1							
124	0.317	0.353	0.750	—	0.200	0.462	0.639
126	0.033	0.015	0.250	—	0.140	0.404	0.056
128	0.067	0.044	—	—	—	—	—
132	0.033	—	—	0.024	—	—	—
134	0.083	0.162	—	0.095	—	—	—
136	0.017	0.059	—	0.048	0.180	—	—
138	0.033	0.088	—	—	0.340	0.135	0.278
140	0.267	0.191	—	0.429	—	—	0.028
142	—	0.029	—	0.048	—	—	—
144	0.100	0.044	—	0.119	0.140	—	—
146	0.017	—	—	0.119	—	—	—
148	—	—	—	0.048	—	—	—
156	—	—	—	0.071	—	—	—
160	0.033	—	—	—	—	—	—
164	—	0.015	—	—	—	—	—
N	60	68	64	42	50	52	36
Pzeb3							
313	0.133	0.088	0.563	—	1.000	1.000	1.000
315	0.183	0.206	0.031	0.190	—	—	—
317	0.183	0.132	0.359	0.071	—	—	—
319	0.150	0.456	—	—	—	—	—
321	0.283	0.029	0.047	0.214	—	—	—

Appendix I *continued*

	Eretmodus cyanostictus				*Tanganicodus* cf. *irsacae*		
Allele length (bp)	KAM	KIK	KOR	KYE	KAM	KAP	KOR
325	0.067	0.044	—	0.524	—	—	—
327	—	0.029	—	—	—	—	—
329	—	0.015	—	—	—	—	—
N	60	68	64	42	50	52	36
UNH002							
154	0.033	—	—	—	—	—	—
158	0.033	0.074	—	—	—	—	—
160	—	0.044	0.063	—	1.000	0.923	0.806
162	0.083	0.074	—	0.548	—	0.077	0.194
164	0.067	0.103	—	—	—	—	—
166	0.033	0.029	0.063	0.024	—	—	—
168	0.133	0.309	0.016	0.143	—	—	—
170	0.117	0.118	0.203	0.048	—	—	—
172	0.117	0.176	0.047	0.024	—	—	—
174	0.017	—	0.016	—	—	—	—
176	0.033	—	0.109	—	—	—	—
178	0.200	0.029	0.047	0.119	—	—	—
180	0.050	0.015	0.063	0.048	—	—	—
182	0.017	—	0.109	—	—	—	—
184	—	—	0.078	—	—	—	—
186	0.033	0.015	0.094	—	—	—	—
188	—	0.015	0.047	0.048	—	—	—
190	—	—	0.016	—	—	—	—
194	—	—	0.016	—	—	—	—
198	0.017	—	—	—	—	—	—
200	0.017	—	—	—	—	—	—
214	—	—	0.016	—	—	—	—
N	60	68	64	42	50	52	36

Appendix II

Alignment of all obtained haplotypes showing the variable sites only. The haplotype numbers as presented in Fig. 1 and their absolute frequency in each population are listed. EMBL/GenBank accession numbers AJ407078–AJ407147

	E. cyanostictus					*T.* cf. *irsacae*				
	KAM	KIK	KAP	KOR	KYE	KAM	KIK	KAP	KOR	111111111111111111111111111222222222222222233333 144777888899112222333334445556666677778001112444557899900223
Haplotype	30	34	6	32	21	25	6	26	18	110914801234131723572356828927913478157812346705782466734737894
1	9	4								GTCATTTCAAGAATCATCATGGTAAAAAATAACATTAATTTTCGTATTGTTTCGGTTTCAAGT
2	6									.....C.....
3	2									A.....T....
4	2	1								.....T.....C...A.....
5	1									.....T....
6	1									.....C.....C.....
7	1									.....C.....C.....
8	1									.....T.....C.....
9	1									.....G.....
10	1									..... G.....T....
11	1									.....C.....
12	1									.....T...A.....C
13	1									.....GTC
14	1									.....G.....C.....C.....
15	1									.....C.....A.....T.....
16		3								.....C.....G.....
17		2								.....C.....A.....A.....
18		2								.....A.....G...
19		2								.....A.....
20		2								.....T.....
21		2								.....T...C.....G.....A.....
22		1								.....TC.....
23		1								.....T...C.....
24		1								.....C.....
25		1								.....AC.....
26		1								.....C.....G.....A..C.A.....
27		1								.....AC.....G...
28		1								.....T...C.....C..G..G.....C.....G..C
29		1								A.....TC.....
30		1								.....CG...C.....
31		1								.....C.....
32		1								.....C.....G..C.....
33		1								.....C.....G.....G...
34		1								.....G.....C.....
35		1								.....T.....
36		1								.....G.....A.....

Appendix II *continued*

	E. cyanostictus					*T.* cf. *irsacae*				
	KAM	KIK	KAP	KOR	KYE	KAM	KIK	KAP	KOR	1111111111111111111111111111222222222222222222233333 14477788888991122223333344455566666777780011124445557899900223
Haplotype	30	34	6	32	21	25	6	26	18	11091480123413172357235682892791347815781234670578246673473789 4
37		1					1			.....C.....C.....
38							2			.....T....C.....C..G..G....C.C....A.....G..C
39						3				.....C.....GT.
40						1				.C.....G.....C.....C.....
41					6					.....CT.....CAACG.....C.....CC...C...C.....CTG.CC
42					3					.....CT...G.....CAAC.....C.....CC...C...C.....CTG.CC
43					2					.....CT...G.....CAACG.....C.....CC...C...C.....CTG.CC
44					2					.....CT.....CAAC.....C.....CC...C...C.....CTG.CC
45					1					.....CT.....CAAC.....C.....CC...C...CA.....CTG.CC
46					1					.....CT...G.....CAACG.....C.....CC...C...CA.....CTG.CC
47					1					.....CT...GG.....CAAC.....C.....CC...C...C.....CTG.CC
48					1					.....CT.....CAAC.....C.....CC..TC...CA.....CTG.CC
49					1					.....CT.....CAACG.G....C.....CC...C...CA.C.....CTG.CC
50					1					.....CT.....CAACG.....C.....CC.C.C...C.....CTG.CC
51					1					.....CT.....CAAC.G....C.....CC...C...CA.C.....CTG.CC
52					1					.....CT.....CAACG.....C.....CC...C...CA....A...CTG.CC
53						19				.CTC....G.A.....TCC.....C...C.....C.....A.....C.TGGT.
54						2				.CTC.....A.....TCC.....C...C.....C.....A.....C.TGGT.
55							2			.CTC.....A...T. TCC.....C.T.C.....C.....A.....C.TGGTC
56							1			.CTC....G.A.....TCC.....C...C.....C.....A.....C.TGGTC
57			3	10						..T.....A.....TCC.....G.CC.T.C.....C....AA.C....C.TGGTC
58				5						..T.....A.....TCC.....CC.T.C.....C....AA.C....C.TGGTC
59			1	5						..T....G.A.....TCC.....G.CC.T..... CC..C....AA.C....C.TGGTC
60				2						..T.....A.....TCC.....G.CC.T.C.....C....AA.C....CCTGGTC
61			1	2						..T....G.A.....TCC.....G.CC.T.C.....C....AA.C....C.TG.TC
62				1						..T.....A.....TCC.....G.CC.T.C.....C.....AA.....C.TGGTC
63				1						..T.....A.....TCC.....G.CC.T.C....C..C....AA.C....C.TGGTC
64				1						..T.....A.....TCC.....G.CC.T.C....C.....AA.A....C.TGGTC
65			1	1						..T.....TCC.....G.CC.T.C....C..C....AA.C....C.TGGTC
66				4				22	9	..T.....A.....TCC.....G.CC.T.C....C..C....AA.C....C.TGGTC
67									8	..T.....TA.....TCC.....G.CC.T.C....C..C....AA.C....C.TGGTC
68								2	1	..TC.....A.....TCC.....G..C...C.....C....AA.A....C.TG.TC
69								1		..T.....A.....TCC.....G.CC.T.C....C..C....AA.....C.TGGTC
70								1		..TCC.....A.....TCC.....G..C...C.....C....AA.A....C.TG.TC

Haplotypes 1–52 clustered in Lineage C whereas haplotypes 53–70 clustered in Lineage D (see Fig. 1).