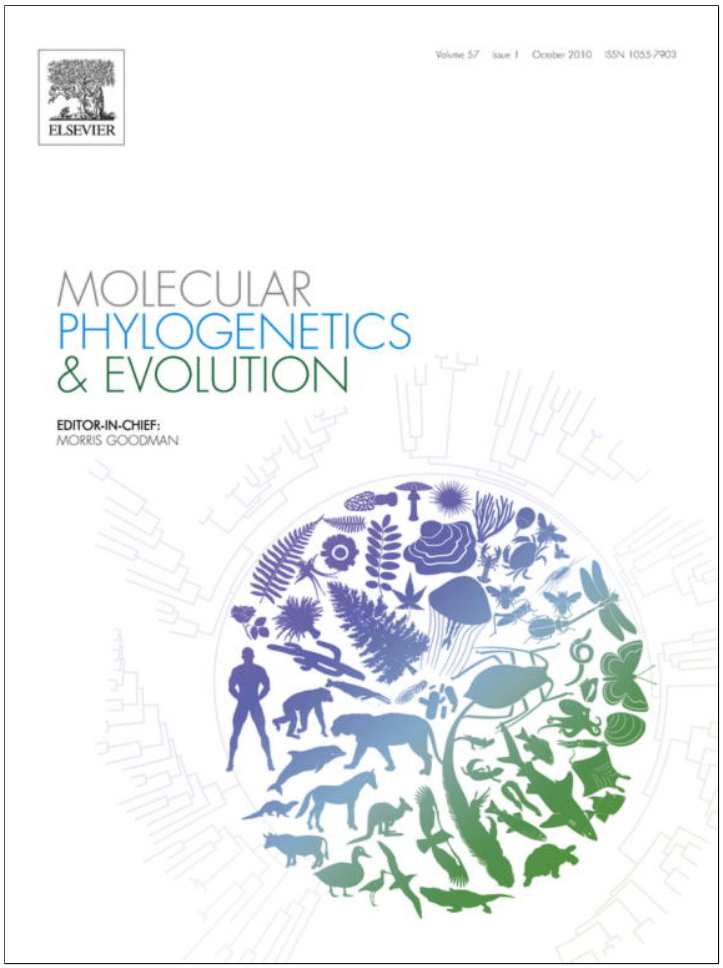

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Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data

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ABSTRACT

Lake Tanganyika comprises a cichlid species flock with substrate-breeding and mouthbrooding lineages. While sexual selection via mate choice on male mating color is thought to boost speciation rates in mouthbrooding cichlids, this is not the case in substrate-breeding lamprologines, which mostly form stable pairs and lack sexual dichromatism. We present a comprehensive reconstruction of the evolution of the cichlid tribe Lamprologini, based upon mtDNA sequences and multilocus nuclear DNA (AFLP) markers. Twelve mtDNA clades were identified, seven of which were corroborated by the AFLP tree. The radiation is likely to have started about 5.3 MYA, contemporarily with that of the mouthbrooding C-lineage, and probably triggered by the onset of deep-water conditions in Lake Tanganyika. Neither the Congo- nor the Malagarazi River species form the most ancestral branch. Several conflicts in the mtDNA phylogeny with taxonomic assignments based upon color, eco-morphology and behavior could be resolved and complemented by the AFLP analysis. Introgressive hybridization upon secondary contact seems to be the most likely cause for paraphyly of taxa due to mtDNA capture in species involving brood-care helpers, while accidental hybridization best explains the para- or polyphyly of several gastropod shell breeders. Taxonomic error or paraphyly due to the survival of ancestral lineages appear responsible for inconsistencies in the genera *Lamprologus* and *Neolamprologus*.

1. Introduction

Lake Tanganyika, the oldest of the East African Great Lakes, comprises by far the greatest diversity of cichlid fishes in terms of morphology, ecology and behavior. Its cichlid species flock includes two substrate-breeding and several mouthbrooding lineages, subdivided into 12–16 tribes (Poll, 1986; Takahashi, 2003; Salzburger, 2009), which are largely supported by comparative morphological and molecular phylogenetic data (reviewed in Koblmüller et al., 2008a). In consequence, Lake Tanganyika contains a polyphyletic conglomerate of lineages which evolved in parallel under the same abiotic influences from a handful of ancient species that once colonized the emerging lake some 9–12 million years ago (MYA; Cohen et al., 1993). Several new lineages formed via adaptive radiation in the lake itself, probably about 5–6 MYA when the proto-lakes fused into one single deep lake with tropical clearwater conditions (Tiercelin and Mondeguer, 1991; Salzburger et al., 2002a; Koblmüller et al., 2008a; but see Genner et al., 2007), and only two lineages colonized the lake at a later stage (Klett and Meyer, 2002; Koch et al., 2007).

With currently more than 80 species described from Lake Tanganyika (Koblmüller et al., 2008a), the lamprologine cichlids dominate Lake Tanganyika's cichlid fauna, comprising about 40% of the lake's species. They are all substrate breeders and form the sister group of an equally diverse lineage of mouthbrooding species, termed the C-lineage (Salzburger et al., 2002a; Clabaut et al., 2005; Koblmüller et al., 2008a). Lamprologines have colonized most lacustrine habitats, but most often live in the littoral zone. Although considered a single tribe (Poll, 1986), lamprologine cichlids encompass tremendous morphological, ecological and behavioral diversity. With respect to size and ecology, some species are large semi-pelagic predators, several are medium sized invertebrate pickers or herbivores, and some are small enough to fit into and live in empty gastropod shells (Sato and Gashagaza, 1997). In fact, shell-breeding represents a highly successful evolutionary strategy, which was suggested to have arisen multiple times during the radiation of the lamprologines (Sturmbauer et al., 1994; Koblmüller et al., 2007a). Remarkably, eight additional lamprologine species are found in the Congo River (Schelly and Stiassny, 2004), and at least one species occurs in the Malagarazi River (De Vos et al., 2001; Schelly et al., 2003). In terms of social organization, some species form pairs (Nakano and Nagoshi, 1990; Sturmbauer et al., 2008) or harems (Yanagisawa, 1987; Walter and

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Trillmich, 1994; Awata et al., 2006; Matsumoto and Kohda, 2007), exhibit sex-role-reversed polyandry (Yamagishi and Kohda, 1996) or are organized in family clans in which the elder offspring contribute to brood care and defense (Taborsky and Limberger, 1981; Taborsky, 1984; Awata et al., 2005; Heg et al., 2005; Heg and Bachar, 2007). Furthermore, several species exhibit alternative male reproductive phenotypes (Sato et al., 2004; Katoh et al., 2005; Ota and Kohda, 2006) and genetic parentage analyses demonstrated helper parasitism in two cooperatively breeding species (Dierkes et al., 1999, 2005; Dierkes et al., 2008; Awata et al., 2005), sneaker fertilization in a gastropod shell breeder (Katoh et al., 2005), unrelated offspring in the nests of another shell-breeding species (Sunobe and Munehara, 2003) and exceptionally high levels of multiple paternity in a socially monogamous species with biparental nest defense (Sefc et al., 2008).

While the monophyly of Poll's tribe Lamprologini is firmly established (Sturmbauer et al., 1994; Stiassny, 1997; Takahashi et al., 1998; Salzburger et al., 2002a), the intra-tribal relationships and taxonomy remain problematic and in need of revision. Morphology-based and molecular approaches have consistently been incongruent, resolving most genera as polyphyletic in molecular phylogenies (Sturmbauer et al., 1994; Salzburger et al., 2002b; Schelly et al., 2006; Day et al., 2007; Koblmüller et al., 2007a; Nevado et al., 2009). The most thorough morphology-based analysis was carried out by Stiassny (1997), in which the "ossified group of lamprologines" was defined in congruence with a mitochondrial phylogeny (Sturmbauer et al., 1994). Ossified group lamprologines posses a sesamoid bone within the labial ligament (see Fig. 3 in Schelly et al., 2006), which is unique among cichlids and perhaps even Perciformes. The definition of the ossified group also highlights the inadequacy of current lamprologine classification, with representatives scattered among the genera *Lamprologus*, *Neolamprologus*, *Lepidiolamprologus*, and *Altolamprologus*. More recently, Takahashi (2003) used a partially different suite of morphological characters to examine relationships among Tanganyikan cichlids, but did not recover the ossified group as a monophyletic assemblage within 10 lamprologine species representing the Lamprologini in a broader taxonomic spectrum. A combined molecular and morphological study of Schelly et al. (2006) addressed the evolution of the genus *Lepidiolamprologus* within the ossified group, and suggested the exclusion of *Lepidiolamprologus cunningtoni* and the inclusion of *Neolamprologus meeli*, *Neolamprologus hecqui*, *Neolamprologus boulengeri* and *Neolamprologus variostigma* plus two undescribed species – one of which has been recently described as *Lepidiolamprologus mimicus* (Schelly et al., 2007) – in the "two-pore" *Lepidiolamprologus*-clade. *Neolamprologus lemairii,* which was suggested to be another potential member of the *Lepidiolamprologus*-clade due to its possession of two pores in the neurocranial lateral line foramina, was resolved outside the clade (Schelly et al., 2006).

As substrate breeders lacking sexual dichromatism the lamprologine cichlids are an interesting test case for the driving forces of speciation, contrasting with the mostly brightly colored males in the maternally mouthbrooding C-lineage (Clabaut et al., 2005). Sexual selection based upon female mate choice on male mating color, which was suggested to be a major driving force of rapid speciation (Terai et al., 2006; Seehausen et al., 2008), can be ruled out as driving force of diversification in lamprologines. On the other hand, lamprologines show other traits, which might be subject to sexual selection such as brood-care helping behavior and size dimorphism. Indeed, previous molecular phylogenetic studies on lamprologines have resulted in surprising findings: While the monophyly of genera defined by Poll (1986) was often corroborated in the mouthbrooding Tanganyikan lineages analyzed so far (Brandstätter et al., 2005; Duftner et al., 2005; Koblmüller et al., 2004, 2005, 2007b; Salzburger et al.; 2002a; Sturmbauer and Meyer, 1993), the taxonomy of the Lamprologini appeared to be much more in conflict with five of Poll's (1986) seven genera being para- or polyphyletic (Sturmbauer et al., 1994; Schelly et al., 2006; Day et al., 2007; Koblmüller et al., 2007a; Nevado et al., 2009). While the relatively few cases of paraphyly or polyphyly of the genera within the Tropheini, Ectodini and Limnochromini were most likely the result of ancient incomplete lineage sorting or convergent evolution of similar morphologies, repeated interspecific hybridization was suggested to be the more likely cause in the Lamprologini (Salzburger et al., 2002b; Schelly et al., 2006; Koblmüller et al., 2007a). Due to the great degree of genetic divergence among conspecifics of *Neolamprologus marunguensis* belonging to different major mtDNA lineages, hybridization was put forward as the most probable explanation, in congruence with the results based on nuclear DNA data (Salzburger et al., 2002b). The same was found for *Lepidiolamprologus nkambae* (Schelly et al., 2006) and in several gastropod shell-breeding species (Koblmüller et al., 2007a; Nevado et al., 2009). This problem was not adequately addressed by the most recent mitochondrial DNA phylogeny of Day et al. (2007), requiring further analyses including nuclear DNA markers.

In the present study we aim to resolve the phylogeny of the Lamprologini more fully by including previously omitted taxa and by applying two different sets of molecular markers - sequences of the mitochondrial NADH dehydrogenase subunit 2 (ND2) genes and a set of 623 AFLP (Amplified Fragment–Length Polymorphism) loci. Furthermore, we attempt to provide a temporal framework for the diversification of this cichlid tribe by applying a Bayesian relaxed molecular clock model to estimate divergence times. Our study includes 72 of the 81described species, plus 5 undescribed species and thus represents the most comprehensive taxon sampling so far. To elucidate the relationships of lake endemics to riverine taxa, our analysis includes two species from the Congo River and another riverine species, *Neolamprologus devosi* (Schelly et al., 2003), which is endemic to the Malagarazi River. To minimize potential confusion due to inadequate generic assignment against the current state of knowledge, we follow Stiassny's (1997) classification, in which the Lamprologini comprise eight genera: *Altolamprologus* Poll, 1986, *Chalinochromis* Poll, 1974, *Julidochromis* Boulenger, 1898, *Lamprologus* Schilthuis, 1891, *Lepidiolamprologus* Pellegrin, 1904, *Neolamprologus* Colombe and Allgayer, 1985, *Telmatochromis* Boulenger, 1898, and *Variabilichromis* Colombe and Allgayer, 1985. In addition, we follow Schelly et al. (2006) by naming all members of the "two-pore" *Lepidiolamprologus*-clade *N. meeli*, *N. hecqui*, *N. boulengeri* and *N. variostigma* as *Lepidiolamprologus*, and by treating *L. cunningtoni* as member of the still problematic genus *Neolamprologus*.

2. Materials and methods

2.1. Taxonomic sampling and DNA extraction

Our study is based on 161 specimens belonging to the cichlid tribe Lamprologini. The complete ND2 gene (1047 bp) was obtained from 91 individuals representing 77 lamprologine species plus 13 outgroup taxa (four species of the tribe Eretmodini plus nine species of the C-lineage sensu Clabaut et al., 2005; based on Salzburger et al., 2002a and Koblmüller et al., 2008a). AFLP data were obtained from 80 individuals representing 42 lamprologine species (71 individuals) plus nine outgroup taxa (two representatives of the tribe Eretmodini plus seven individuals representing three species of the C-lineage). Due to insufficient DNA quality not all species included in the mitochondrial dataset could be included in the AFLP data. Note that only a few representatives of the ossified group lamprologines have been used for AFLP finger-

printing because a previous study (Koblmüller et al., 2007a) explicitly dealt with the phylogenetic relationships (mtDNA + AFLPs) within the ossified group lamprologines. Most of the specimens were sampled during several field expeditions from 1995 to 2007, and some additional samples were obtained from the aquarium trade (Appendix A). Voucher specimens are stored at the Department of Zoology, University of Graz, Austria and the Royal Africa Museum in Tervuren, Belgium. For all specimens, fin clips were taken and preserved in 99% ethanol. For DNA extraction we applied a proteinase *K* digestion followed by protein precipitation with ammonium acetate.

2.2. mtDNA analysis

Polymerase chain reaction (PCR), purification of PCR products, and sequencing followed the protocol described in Duftner et al. (2005). The primers used for PCR and sequencing were Met, ND2.2A, Trp (Kocher et al., 1989) and ND2.T-R (Duftner et al., 2005) for ND2. DNA fragments were purified with SephadexTM G-50 (Amersham Biosciences) following the manufacturer's instruction and subsequently visualized on an ABI 373 or an ABI 3130xl automated sequencer (Applied Biosystems). All sequences are available from GenBank under the accession numbers listed in the Appendix A.

DNA sequences were aligned by eye using the Sequence Navigator software (Applied Biosystems). To assess the overall phylogenetic signal we performed a likelihood-mapping analysis (Strimmer and von Haeseler, 1997), using TREE–PUZZLE 5.1 (Schmidt et al., 2002). Phylogenetic inference was based on neighbor joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI).

NJ, MP and ML were performed in PAUP* version 4.0b10 (Swofford, 2002). To assess the degree of saturation of transition (*ti*) and transversion (*tv*) mutations at each codon position, we plotted the number of mutations against pairwise uncorrected *p*-distances (not shown). Based on the estimated *ti*/*tv* ratio inferred from these pairwise comparisons we derived a weighting scheme for a weighted MP analysis: 30:2 for third codon positions of two and three fold degenerate amino acids, 5:2 for third codon positions of four fold degenerate amino acids, 30:24 for second codon positions and 30:12 for first codon positions. C/T substitutions at the first codon position of leucine were treated as a fifth base and were down-weighted to the same weight as transitions at third codon positions. For NJ and ML analysis, we applied the best-fitting substitution model selected by Modeltest 3.06 (Posada and Crandall, 1998): TrN + I + G (Tamura and Nei, 1993) with nucleotide frequencies $A = 0.2640$, $C = 0.3656$, $G = 0.1220$, $T = 0.1484$; proportion of invariable sites (I) = 0.3834; gamma shape parameter (α) = 1.1482; and *R*-matrix $A \leftrightarrow G$, $A \leftrightarrow T$, $C \leftrightarrow G$, $G \leftrightarrow T = 1.0000$, $A \leftrightarrow G = 20.2677$, $C \leftrightarrow T = 6.7919$. Heuristic tree searches under MP and ML criteria applied random addition of taxa and TBR branch swapping (1000 replicates for MP and 100 replicates for ML). Statistical support for the resulting topologies was assessed by bootstrapping (1000 pseudo-replicates for NJ and MP) and quartet puzzling (25,000 random quartets for ML). For BI, performed in MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001), data were partitioned by codon position. Rate heterogeneity was set according to a gamma distribution with six rate categories (GTR model; Yang, 1994) for each data partition. Posterior probabilities were obtained from Metropolis-coupled Markov chain Monte Carlo simulations (2 independent runs; 10 chains with 3 million generations each; chain temperature: 0.2; trees sampled every 100 generations). A 50% majority-rule consensus tree was constructed after a 1 million generation burn-in to allow likelihood values to reach stability (chain stationarity and run parameter convergence were checked using Tracer v1.4; Rambaut and Drummond, 2008). To assess whether the topologies obtained by the different tree building algorithms differed significantly we performed Shimodaira–Hasegawa (SH) tests (Shimodaira and Hasegawa, 1999; full optimization; 1000 bootstrap replicates) in PAUP*.

Despite some inherent problems (e.g. Pulquério and Nichols, 2007), time estimates based on molecular data do provide an approximate framework to put diversification events in a temporal context. Divergence times were estimated within a Bayesian MCMC framework in the program BEAST v1.4.8 (Drummond and Rambaut, 2007), including the above-mentioned taxa plus additional representatives of the tribe Haplochromini (including additional taxa of the tribe Tropheini, which is nested within the Haplochromini (see Salzburger et al., 2005; Koblmüller et al., 2008b)), and *Boulengerochromis microlepis*, *Bathybates leo*, *Hemibates stenosoma* and *Telotrematocara macrostoma* as outgroups, justified by previously published evidence regarding the phylogenetic relationships among Lake Tanganyika's cichlid tribes (reviewed in Koblmüller et al., 2008a). The inclusion of these additional taxa was necessary due to a lack of potential calibration points within the Lamprologini. Divergence times were calculated as 95% highest posterior density (HPD) intervals on a time-measured phylogeny. We employed the SRD06 two-partition codon-specific rates model of nucleotide evolution, which has fewer parameters than the GTR + I + G model, but has been shown to provide a better fit for protein coding sequence data (Shapiro et al., 2006) and implemented a relaxed molecular clock with log-normally autocorrelated rates among branches (Drummond et al., 2006). We used default settings for all priors except for the tree prior which was set to Yule Process (speciation) as suggested by Drummond et al. (2007). Monophyly of clades was not enforced, except for those used as calibration points, and operators were auto-optimized. As calibration points we applied the age of 5–6 MY for the formation of a truly lacustrine habitat with clear- and deep-water conditions in Lake Tanganyika (Tiercelin and Mondeguer, 1991; Lezzar et al., 1996; Cohen et al., 1997), constraining: (i) the time of the most recent common ancestor (MRCA) of the C-lineage encompassing the majority of mouthbrooding Lake Tanganyika cichlid tribes (Clabaut et al., 2005), and (ii) the time for the MRCA of the tribe Lamprologini, assuming that the onset of their diversification, the "primary radiation" in Lake Tanganyika, was coincident with that of the C-lineage (Salzburger et al., 2002a; Koblmüller et al., 2008a); (iii) a minimum age of 1.1 MY for the split of the Congo River lamprologines, based on the time window for a Lukuga-connection between Lake Tanganyika and the Congo system (Lezzar et al., 1996; Cohen et al., 1997); (iv) a maximum age of 0.57–1 MY for the split between the utaka and mbuna cichlids, based on the age of the refilling of Lake Malawi (Delvaux, 1995; Sturmbauer et al., 2001). A preliminary run was used for parameter optimization. The final analysis was run for 10^7 generations, with trees sampled every 1000 generations. The log file was analyzed using Tracer v1.4 (Rambaut and Drummond, 2008) to determine the appropriate burn-in (10^6 generations), which was discarded from the log and tree file. The post burn-in effective sample sizes (ESSs) for all parameters were >300, indicating that the log file accurately represented the posterior distribution (Kuhner, 2009). Divergence times were calculated from the post burn-in results and TreeAnnotator v1.4.8 (a module in the BEAST program package) was used to compute a maximum-clade-credibility tree, which was visualized in FigTree v1.2.2 (Rambaut, 2009). In order to check whether the posterior probabilities were actually affected by the data, we also ran analyses in which BEAST only sampled from the prior distribution (Drummond et al., 2006). To test for the effect of calibration points on divergence time estimates, we performed two additional analyses with different combinations of these calibration points (Table 2). Recently, alternative age estimates for the East African cichlid radiations have been proposed (Genner et al., 2007). Apply-

ing these calibration points for the Lake Tanganyika radiation yielded age estimates that are at odds with geology-based lake history data (see Koblmüller et al., 2008a, b). To evaluate the alternatives, we applied two alternative calibration schemes: We conducted a second analysis in which we assumed an alternative age estimate of 1.1–3.5 MY for the split of the Congo River lamprologines, based on the time window for a Lukuga-connection between Lake Tanganyika and the Congo system and constrained the primary Tanganyika radiation (in our case the MRCA of the C-lineage) to an age of 9–12 million years. This was based on the assumption that the primary Tanganyika radiation had taken place at the onset of the Lake formation 9–12 MYA as a consequence of allopatric diversification in a series of small shallow lakes in the area currently occupied by the Congo River system and Lake Tanganyika (Tiercelin and Mondeguer, 1991; Cohen et al., 1993). This assumption implies that the secondary radiation observed in most Lake Tanganyika cichlid tribes (Koblmüller et al., 2004, 2005, 2007a,b, 2010; Duftner et al., 2005) coincided with the formation of a real lacustrine habitat with deep-water conditions about 5–6 MYA, compatible with the fossil calibrated diversification scenario discussed by Genner et al. (2007). As a third alternative calibration, we applied a normally distributed prior with a mean of 30 MYA (S.D., 3.0 MY; to account for some degree of uncertainty in this age estimate) for the MRCA of the C-lineage, as was proposed by Genner et al. (2007) based on a presumed Gondwanan origin of the family Cichlidae.

2.3. AFLP analysis

Restriction digestion, pre-selective and selective PCR followed the protocol described in Egger et al. (2007). For selective amplification we used the following eight primer combinations: EcoRI-ACA/MseI-CAA, EcoRI-ACA/MseI-CAG, EcoRI-ACA/MseI-CAC, EcoRI-ACA/MseI-CAT, EcoRI-ACT/MseI-CAA, EcoRI-ACT/MseI-CAG, EcoRI-ACT/MseI-CAC, EcoRI-ACT/MseI-CAT. Selective PCR products were visualized on an ABI 3100xl automated sequencer (Applied Biosystems) along with an internal size standard (GeneScan-500 ROX, Applied Biosystems).

Raw fragment data were analyzed using GENEMAPPER v.3.7 (Applied Biosystems). We found automated scoring problematic with respect to fragments that showed gradual intensity differences between samples. Because any detection threshold has to be arbitrary in such cases, presence or absence of peaks (assumed to represent homologous fragments) was scored by eye within a range of 100–500 bp (only clearly distinguishable and unambiguous peaks were scored) and assembled as a binary matrix in GENEMAPPER. In a few cases, fragments were scored as missing data when character states could not be determined unambiguously. It is clear that manual scoring yields a lower number of marker bands than automated scoring, but data quality is substantially improved by this approach. Matrices from the different primer combinations were combined into one data set yielding a data matrix of 80 individuals × 623 loci.

A NJ tree based on Nei and Li's (1979) distances was constructed in PAUP* and bootstrap values from 1000 pseudo-replicates were used as a standard measure of confidence in the reconstructed tree topology.

3. Results

3.1. mtDNA phylogeny

Likelihood mapping yielded 91.2% fully resolved quartets (Fig. 1), indicating a strong phylogenetic signal in the dataset. Pairwise sequence divergence (uncorrected *p*-distance) between lamprologine species ranged from 0.2% to 13.4%.

Whereas NJ, ML and BI analyses yielded highly congruent results with only minor differences with respect to the tree building algorithm used, SH tests revealed a significantly lower likelihood value for the MP topology (Table 1). Only the best tree (BI) – based on the likelihood criterion – is shown in Fig. 2.

Fig. 3 shows a 75% majority-rule consensus tree based on the NJ, MP, ML and BI topologies. We present a 75% majority rule and not a strict consensus tree because of the significantly deviating MP topology (see above), which is equivalent to the strict consensus of the outcome of the remaining three tree building algorithms. Of the 1047 bp of ND2, 561 bp showed variation and 449 bp were parsimony- informative. The maximum parsimony analysis resulted in 62 most parsimonious trees (weighted tree length = 15,691 steps; CI excluding uninformative sites = 0.3994; RI = 0.6920; trees not shown). Neighbor-joining, maximum likelihood and Bayesian inference yielded the same tree topology. Our new phylogeny is fully compatible with earlier works (Sturmbauer et al., 1994; Schelly et al., 2006; Day et al., 2007; Koblmüller et al., 2007a). All four tree building algorithms revealed strong support for the monophyly of the Lamprologini. Within the Lamprologini, 12 clades emerged: clade I comprising two subclades, one with *Neolamprologus nigriventris*, *Neolamprologus mustax*, *Neolamprologus cylindricus*, and the second subclade with *Neolamprologus gracilis*, *Neolamprologus savoryi*, *Neolamprologus olivaceous*, *Neolamprologus niger* and *N. marunguensis* (clade I in Fig. 3); clade II comprising the very species-rich ossified group with *Neolamprologus similis* as its most ancestral split, followed by a subclade with *Lamprologus ornatipinnis*, *Lamprologus kungweensis*, *Lamprologus laparogramma* and *Lamprologus signatus*, another subclade with the *Lamprologus speciosus*, *Neolamprologus calliurus* and *Neolamprologus brevis*, a subclade with *Neolamprologus multifasciatus*, the subclade with *Neolamprologus leloupi*, the subclade with *Lamprologus lemairii* and *Neolamprologus caudopunctatus*, the subclade comprising *Lamprologus meleagris*, the subclade with *L. nkambae*, the subclade containing *Lamprologus ocellatus*, three species of *Altolamprologus*, *Lamprologus callipterus* and *Neolamprologus fasciatus*, and the subclade comprising the members of the "two-pore" *Lepidiolamprologus*-clade *Lepidiolamprologus profundicola*, *Lepidiolamprologus elongatus*, *Lepidiolamprologus variostigma*, *Lepidiolamprologus kendalli*, *Lepidiolamprologus attenuatus*, *L.* sp. nov. "hecqui-boulengeri", *Lepidiolamprologus boulengeri*, *Lepidiolamprologus hecqui* and *Lepidiolamprologus meeli* (clade II); clade III comprising *Neolamprologus tretocephalus* and *Neolamprologus sexfasciatus*; clade IV with *Neolamprologus toae*; clade V with *Variabilichromis moorii*; clade VI with *Neolamprologus prochilus*, *Neolamprologus obscurus*, *Neolamprologus leleupi* and *Neolamprologus longior*, clade VII comprising *Neolamprologus buescheri*, clade VIII with *Neolamprologus cunningtoni*, *Neolamprologus tetracanthus* and *Neolamprologus modestus*, clade IX with *Neolamprologus furcifer*; clade X with *Neolamprologus christyi*; clade XI comprising four *Chalinochromis* species with *Julidochromis ornatus*, *Julidochromis transcriptus* and *Julidochromis dickfeldi* in paraphyletic grouping; and the species-rich clade XII with five subclades, the first with *Neolamprologus falcicula* (clade XII.1), the second containing *Neolamprologus petricola*, *Telmatochromis vittatus*, the two Congo River species *Lamprologus teugelsi* and *Lamprologus congoensis*, *Neolamprologus mondabu*, *N.* sp. nov. "eseki", the Malagarazi River species *N. devosi* and *Telmatochromis temporalis* (clade XII.2), the third subclade comprising the brood-care helper species *Neolamprologus pulcher*, *N. gracilis*, *N. marunguensis*, *Neolamprologus helianthus*, *Neolamprologus brichardi* and *Neolamprologus splendens* (clade XII.3), the fourth subclade with *Neolamprologus walteri*, *Julidochromis marlieri* and *Julidochromis regani* (clade XII.4), and the fifth subclade comprising various genotypes of *N. savoryi*, *Telmatochr-*

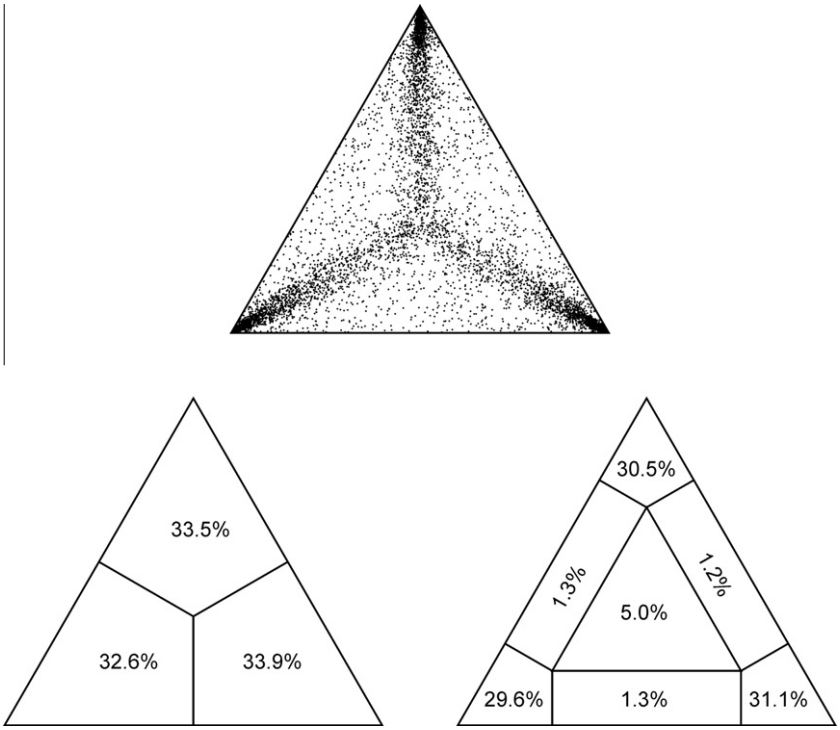

Fig. 1. Results from the likelihood mapping analysis of the ND2 dataset, indicating the presence of a strong phylogenetic signal.

Table 1
Comparison of alternative phylogenetic hypotheses.

Tree	$-\ln L$	$\Delta -\ln L$	P
NJ	14248.05672	39.89702	0.157
MP	14358.34803	150.18833	<0.001
ML	14209.70277	1.54307	0.815
BI	14028.15970	Best	

Shimodaira–Hasegawa (SH) tests (Shimodaira and Hasegawa, 1999) were used to determine whether NJ, MP, ML and BI topologies differed significantly under a maximum likelihood criterion.

omis bifrenatus, *Telmatochromis dhonti*, and *T. temporalis* (clade XII.5). Fig. 2b depicts the Bayesian tree; NJ and ML yielded highly similar topologies. The branching order of the 12 clades recovered was as follows: Clades VI–XII were always resolved as monophylum (VI, (VII, (VIII, (IX, (X, (XI, XII))))). This monophylum – albeit with slightly different branching order (VI, (X, (IX, (VIII, (XI, XII))))) – was also found in MP (trees not shown), when *N. buescheri* (clade VII) was excluded (it was placed inside clade I in MP).

3.2. AFLP phylogeny

For 80 taxa (representing 47 species) AFLPs could successfully be amplified. Seven out of the 12 clades corresponded to those identified on the basis of mtDNA sequences, as depicted in Fig. 4: clade II comprising the ossified group; clade III comprising *N. tretocephalus* and *N. sexfasciatus*; clade IV with *N. toae*; clade V with *V. moorii*; clade VII comprising *N. buescheri*; clade IX with *N. furcifer*; clade X with *N. christyi*; and clade XI comprising four *Chalinochromis* species with *J. ornatus*, *J. transcriptus* and *J. dickfeldi*. In the species-rich clade XII with five subclades, three of the five subclades were retained: subclade XII.3 comprising *N. helianthus*, *N. olivaceous*, *N. savoryi*, *N. marunguensis*, *N. brichardi* and *N. pulcher*, was retained as well as subclade XII.4 with *J. marlieri* and *J. regani*, and subclade XII.5 comprising all analyzed individuals of the three species of the genus *Telmatochromis*, *T. bifrenatus*, *T. dhonti*, and *T. temporalis*. Subclade XII.1 with *N. falcicula* was not corroborated due to insufficient DNA quality, subclade XII.2 represented by *N. petricola*, *N. mondabu* and the Congo River species *L. teugelsi*, was split into two clades. Clade VI represented by *N. prochilus*, *N. obscurus* and *N. leleupi* was split into three separate clades, clade VIII with *N. cunningtoni*, *N. tetracanthus* and *N. modestus* was split into one clade comprising *N. cunningtoni* and *N. tetracanthus*, a second with *N. modestus*. In summary, the AFLP tree was highly compatible with the mtDNA phylogeny by corroborating 7 of the 12 clades with good bootstrap support. The remaining 5 mtDNA clades were subdivided. As in the mtDNA phylogeny, most branches between clades were short. In contrast to the mtDNA tree, however, all species recovered as polyphyletic in the mtDNA phylogeny were monophyletic in the AFLP tree. Moreover, the genus *Telmatochromis*, which was scattered in the mtDNA tree, was monophyletic in the AFLP analysis, as were the *Neolamprologus* species with brood-care helper behavior of clade XII.3. The paraphyletic placement of the genus *Julidochromis* was retained, corroborating the close relationship of *J. ornatus*, *J. transcriptus* and *J. dickfeldi* with the genus *Chalinochromis*.

3.3. Mitochondrial phylo-chronology

The chronogram for the radiation of the lamprologine cichlids resulting from a relaxed molecular clock analysis with BEAST and applying four calibration points ((i) MRCA of the C-lineage; (ii) MRCA of the Malawi cichlid species flock; (iii) MRCA of the Lamprologini; (iv) time window for the colonization of the Congo system via the Lukuga River) is shown in Fig. 5 (chronograms based on alternative sets of calibration points are shown in Supplementary Fig. 1–4). For all calibration nodes in all analyses, mean posterior estimates were close to prior node ages (but usually with smaller HPD intervals), indicating that the combinations of calibration points are reasonably concordant (Sanders and Lee, 2007). The mean rate covariance was slightly negative in all analyses, with

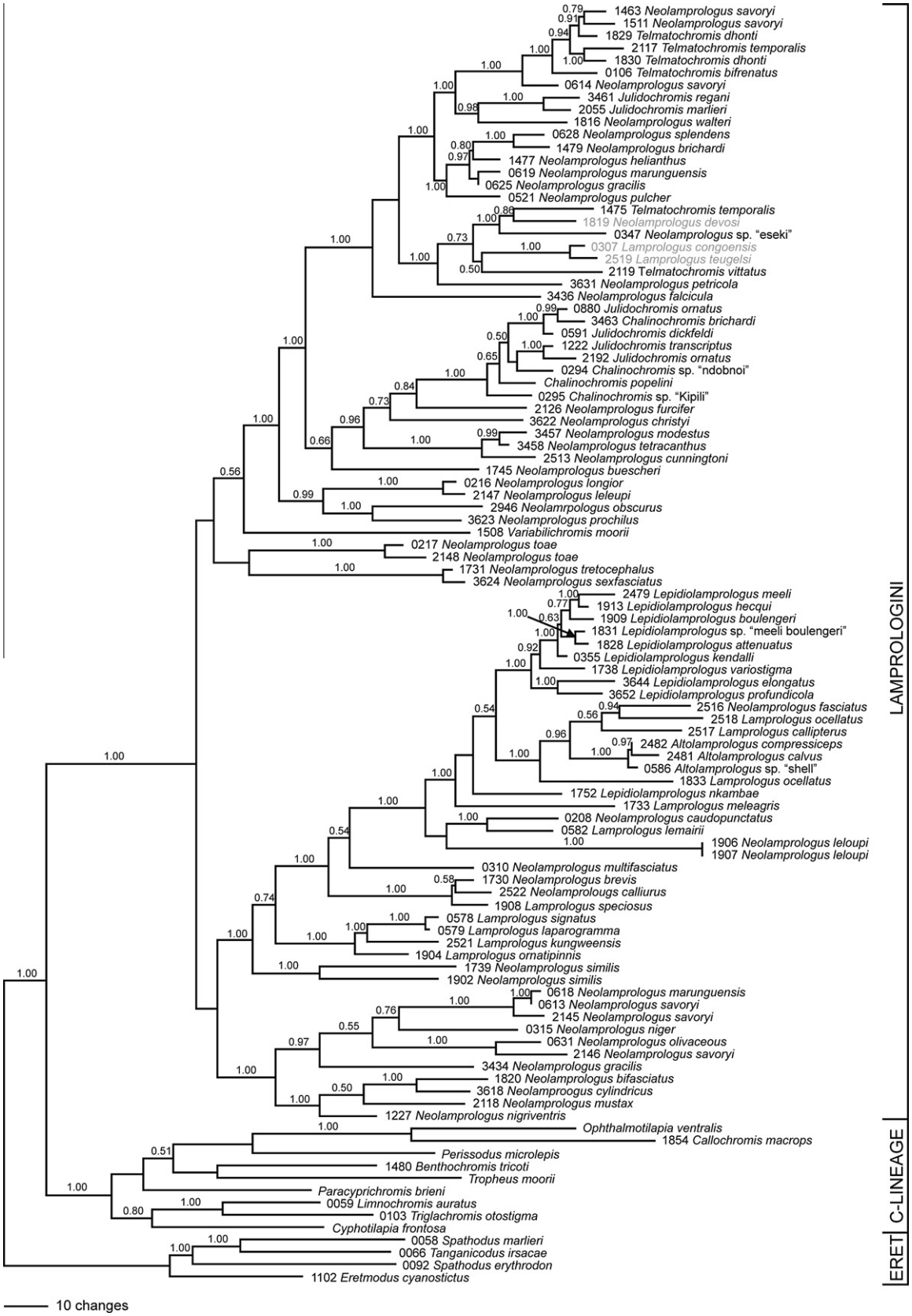

Fig. 2. Bayesian 50% majority-rule consensus tree of the ND2 dataset. Only posterior propabilities >0.50 are shown. Riverine lamprologine taxa are shaded in grey.

its 95% HPD interval spanning zero (not shown), indicating that branches with fast and slow substitution rates are next to each other, meaning that there is no strong evidence for autocorrelation of rates in the phylogeny (Drummond et al., 2006). All calibration sets yielded similar significant but not exceptionally high levels of rate heterogeneity among lineages (mean coefficients of branch

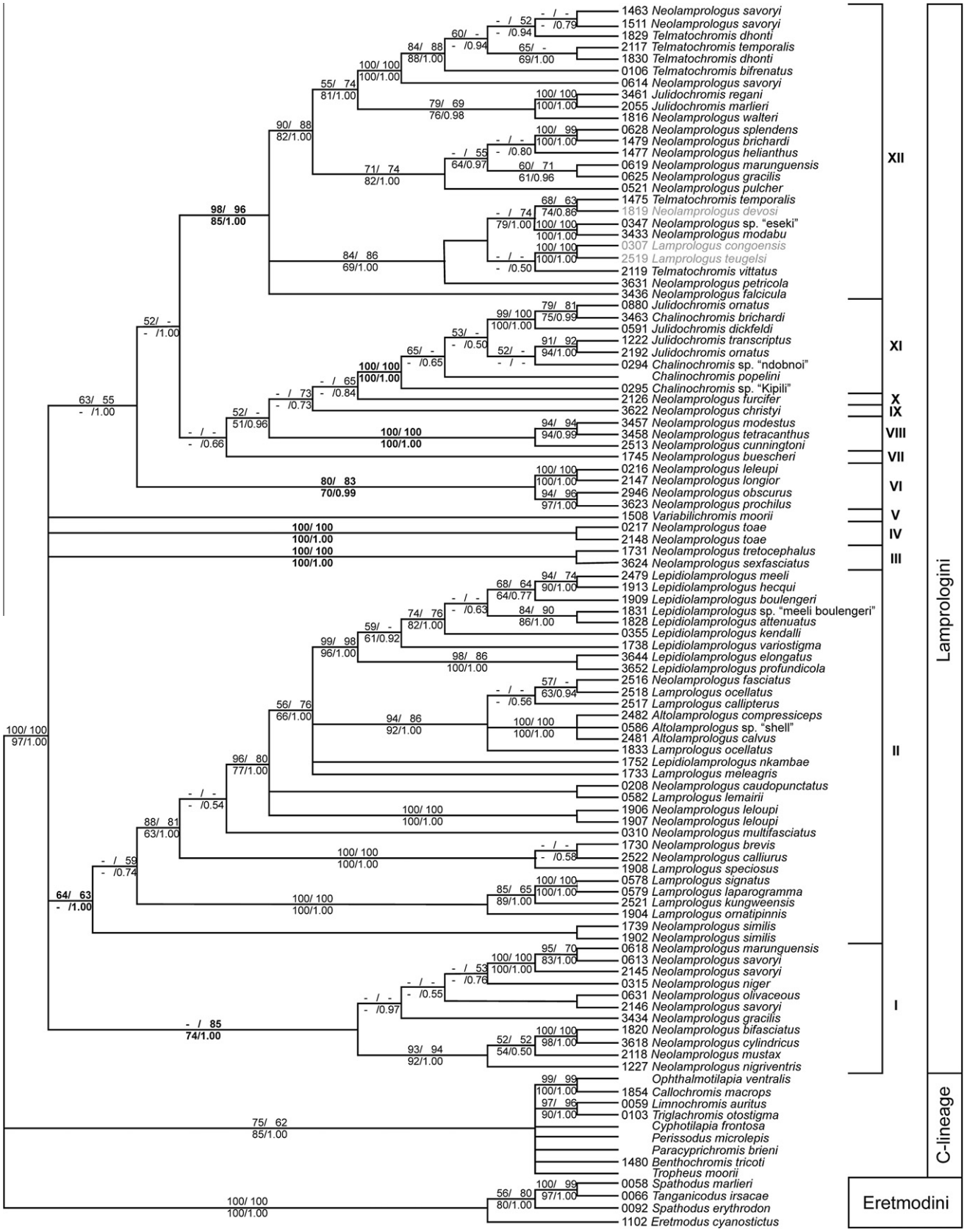

Fig. 3. 75% Majority-rule consensus tree of the NJ, ML, BI and strict consensus of 62 most parsimonious trees, representing the phylogenetic relationships of the Lamprologini based on the complete ND2 gene. Only bootstrap values >50 and posterior probabilities >0.50 are shown. Roman numbers indicate major, well supported mtDNA lineages (corresponding bootstrap values and posterior probabilities are printed in bold lettering). Riverine lamprologine taxa are shaded in grey.

rate variation, 0.32–0.34; Table 2), thus justifying the use of a relaxed clock model (Drummond et al., 2007). Mean substitution rates varied between 0.01597 and 0.01794 substitutions per site per MY among the analyses assuming that the primary radiation

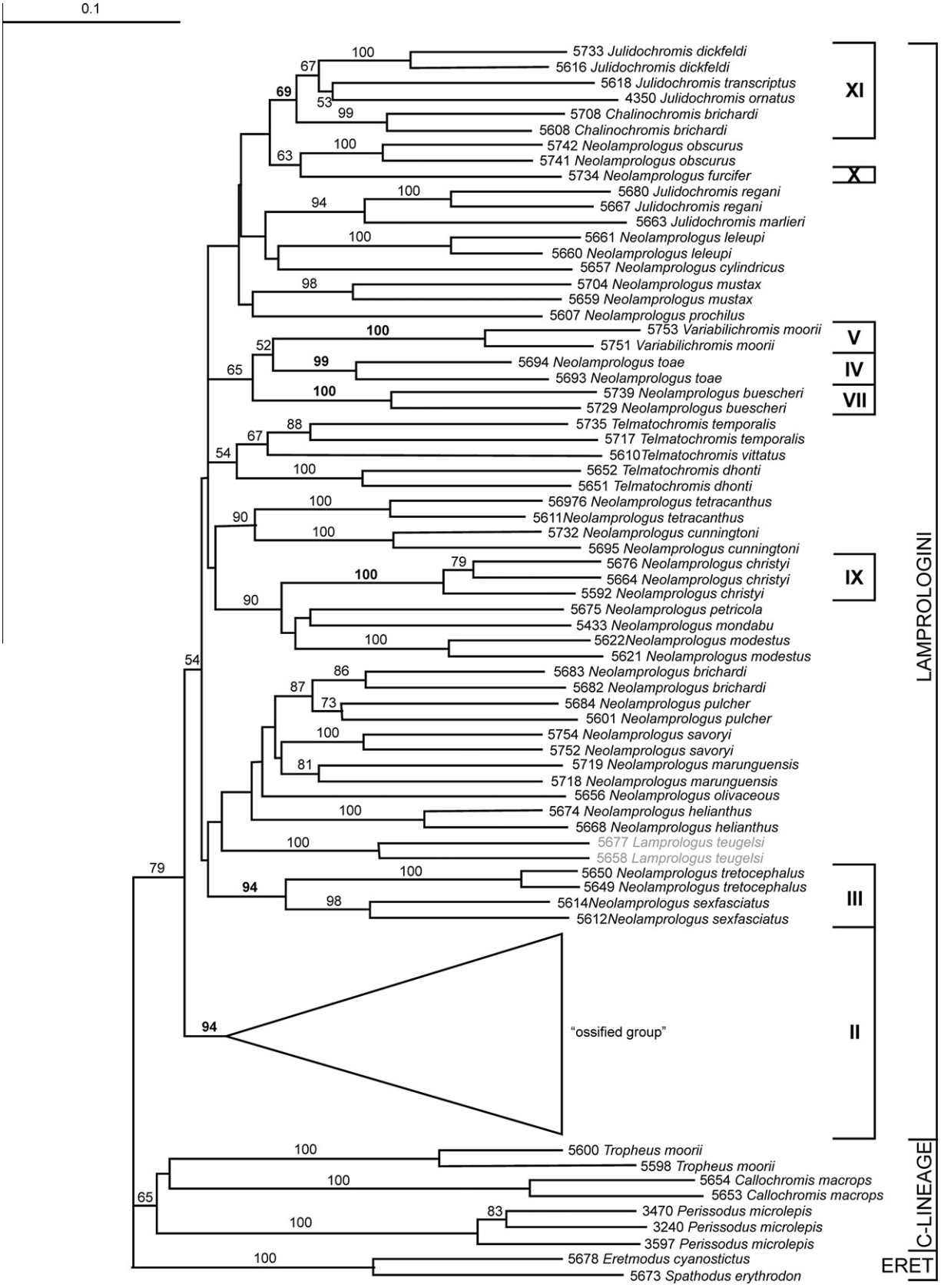

Fig. 4. NJ tree (Nei and Li distances Nei and Li, 1979)) based on 623 AFLP loci. Only bootstrap values >50 are shown. Riverine lamprologine taxa are shaded in grey.

(Salzburger et al., 2002a) coincided with the formation of a real lacustrine habitat 5–6 MYA (Table 2). Analyses assuming ages of 9–12 and 30 MY for the diversification of the C-lineage yielded mean substitution rates of 0.00919 and 0.00317 substitutions per site per MY, respectively (Table 2). We emphasize that our divergence time estimates should be regarded as approximate, because

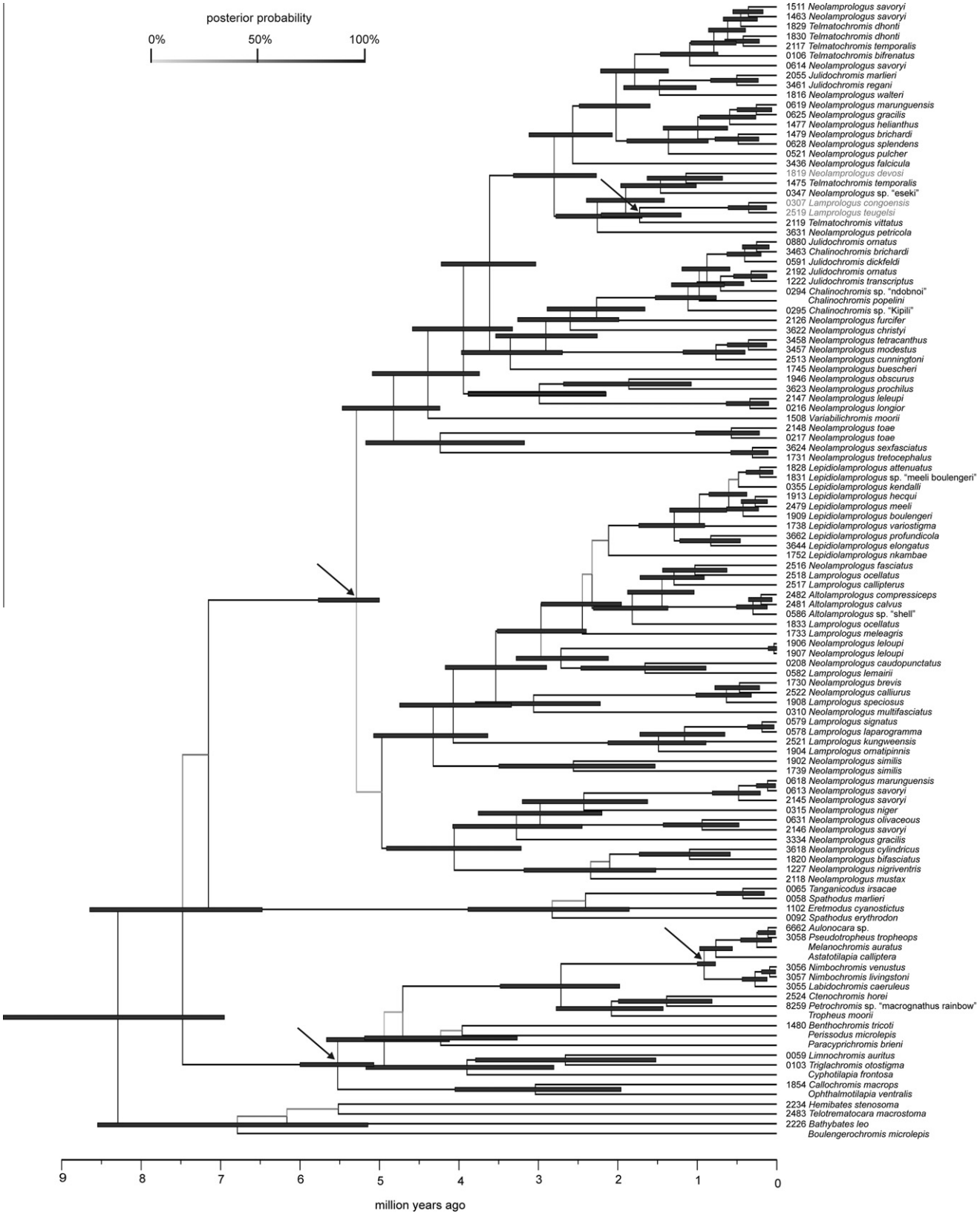

Fig. 5. A chronogram of the diversification of the Lamprologini based on complete ND2 sequences, assuming that the diversification of both the C-lineage and the Lamprologini started in the course of the so-called "primary radiation", which was proposed to have coincided with the formation of tropical clearwater habitat with deep-water conditions 5–6 MYA (Salzburger et al., 2002a); assuming a maximum age of 0.57–1 MY for the Lake Malawi cichlid species flock, based on the age of the refilling of Lake Malawi (Delvaux, 1995; Sturmbauer et al., 2001); and assuming an age of 1.1–3.5 MY for the split of the Congo-lamprologines, based on the time window for a Lukuga-connection between Lake Tanganyika and the Congo system (Lezzar et al., 1996; Cohen et al., 1997). Chronograms for alternative diversification scenarios are shown in Supplementary Fig. 1–4. Divergence time estimates are represented as the mean node height of the 95% highest posterior density (HPD) interval from a BEAST maximum-clade-credibility tree. Grey bars span the 95% HPD for each well supported node. No bars are assigned to nodes with low posterior probability. Calibration points are marked by arrows. Riverine lamprologine taxa are shaded in grey.

Table 2
Testing the influence of particular calibration points on posterior estimates of divergence times, with mean rate and coefficient of variation for each scenario.

Calibrations used	MVhL-clade*	Posterior estimates of calibration points				Mean rate ($\times 10^{-3}$)	Coefficient of variation
		C-lineage	Lake Malawi flock	Lamprologini	Congo/Lake Tanganyika		
C-lineage (5–6)/LM (0.57–1)/ Lamprologini (5–6)/Congo (1.1–3.5)	7.42 (6.42–8.58)	5.51 (5.06–6.00) 5.39 (5.00–5.89)	0.92 (0.78–1.00) 0.84 (0.62–1.00)	5.28 (5.00–5.76) 5.53 (5.07–6.00)	1.68 (1.18–2.15) 1.69 (1.10–2.77)	15.97 (13.91–17.97)	0.34 (0.26–0.43)
C-lineage (5–6)/LM (0.57–1)/ Congo (1.1–3.5)	6.72 (5.57–8.00)	5.35 (5.00–5.86) 5.43 (5.00–5.92)	0.90 (0.74–1.00) 0.84 (0.62–1.00)	4.55 (3.68–5.52)	1.50 (1.10–1.91) 1.77 (1.10–2.94)	17.94 (14.63–21.12)	0.32 (0.24–0.41)
C-lineage (5–6)/LM (0.57–1)	6.75 (5.57–8.11)	5.35 (5.00–5.87) 5.41 (5.00–5.91)	0.90 (0.74–1.00) 0.84 (0.62–1.00)	4.57 (3.61–5.54)	1.50 (1.01–2.02)	17.87 (14.55–21.20)	0.33 (0.25–0.42)
C-lineage (9–12)/ Congo (1.1–3.5)	13.06 (10.37–16.10)	10.14 (9.00–11.65) 10.35 (9.00–11.80)	2.34 (1.40–3.40)	8.87 (6.90–11.06)	2.82 (2.10–3.50) 2.12 (1.10–3.30)	9.19 (7.27–11.20)	0.33 (0.24–0.41)
C-lineage (30)	38.00 (27.89–49.01)	29.07 (23.21–35.12)	6.83 (4.00–10.12)	25.96 (18.49–33.58)	8.50 (5.49–11.99)	3.17 (2.33–4.06)	0.33 (0.24–0.41)

We ran three independent analyses in BEAST using combinations of different sets of calibration points (uniform priors): (i) 5–6 MY for the most recent common ancestor (MRCA) of the cichlids assigned to the C-lineage (encompassing the majority of mouthbrooding Lake Tanganyika cichlid tribes; Clabaut et al., 2005), assuming that this "primary radiation" (Salzburger et al., 2002a) coincided with the formation of a truly lacustrine habitat with deep-water conditions in Lake Tanganyika (Tiercelin and Mondeguer, 1991; Lezzar et al., 1996; Cohen et al., 1997); (ii) a maximum age of 0.57–1 MY for the split between the utaka and mbuna cichlids, based on the age of the refilling of Lake Malawi (Delvaux, 1995; Sturmbauer et al., 2001); (iii) an age of 5–6 MYA for the MRCA of the tribe Lamprologini, assuming that the onset of their diversification also coincided with the formation of a truly lacustrine habitat with deep-water conditions in Lake Tanganyika; (iv) a minimum age of 1.1 MY for the split of the Congo-lamprologines, based on the time window for a Lukuga-connection between Lake Tanganyika and the Congo system (Lezzar et al., 1996; Cohen et al., 1997). In a fourth run we assumed an age of 9–12 MY for the MRCA of the C-lineage, compatible with the fossil calibrated diversification scenario discussed by Genner et al. (2007). In a fifth run we applied a normally distributed prior with a mean of 30 MYA (S.D., 3.0 MY) for the MRCA of the C-lineage, based on Genner et al.'s (2007) Gondwanan origin scenario for the family Cichlidae.

In order to check whether the posterior probabilities were actually affected by the data, we also ran analyses in which BEAST only sampled from the prior distribution (only for analyses with at least two calibration points). Estimates based on priors only are given below the estimates using the data.

Age estimates are indicated in MY. Numbers in parentheses represent the 95% highest posterior density (HPD) interval.

* The MVhL clade includes the C-lineage, the Lamprologini and the Eretmodini (Takahashi et al., 2001).

the 95% HPD intervals of adjacent nodes are large and often overlapping.

Assuming that the diversification of both the C-lineage and the Lamprologini started in the course of the so-called "primary radiation", which was proposed to have coincided with the formation of tropical clearwater habitat with deep-water conditions 5–6 MYA (Salzburger et al., 2002a), the Lamprologini were estimated to have diverged from the Eretmodini and the C-lineage approximately 7.5 MYA. The onset of diversification within the Lamprologini was estimated to ~5.3 MYA. Note that this estimate was heavily constrained by applying a uniform prior of 5–6 MYA for this particular node. In the course of this first cladogenesis event at the base of the lamprologine radiation, clades I and II (ossified group), as well as the ancestor of clades III–XII emerged. The ancestor of the species poor clades III (*N. toae*) and IV (*N. sexfasciatus* + *N. tretocephalus*) branched off ~4.8 MYA and the split between these two lineages happened ~4.2 MYA. The next clade to diverge was clade V (*V. moorii*) at ~4.4 MYA, followed by clade VI ~4 MYA. The split between the ancestor of clades VII–XI and clade XII happened ~3.7 MYA. The mostly species poor lineages VII–XI, diverged in subsequent order ~3.4, ~2.9, ~2.6 and ~2.3 MYA. The most recent common ancestor (MRCA) of the *Chalinochromis*/*Julidochromis* species in clade XI dates back to ~1.2 MYA. The split of the Congo River lamprologines from the closet related Lake Tanganyika species (*T. vittatus*) was estimated at ~1.7 MYA, which lies well within the possible time window of 1.1–3.5 MYA for the connection of Lake Tanganyika with the Congo system (Lezzar et al., 1996; Cohen et al., 1997). *N. devosi*, which occurs in the Malagarazi River, diverged from its Lake Tanganyika sister taxon ~1.2 MYA. Taking together this series of cladogenesis events, the radiation of the Lamprologini seems to have happened more gradually than that of Lake Tanganyika's mouthbrooding cichlid lineages (Salzburger et al., 2002a; Sturmbauer et al., 2003; Brandstätter et al., 2005; Koblmüller et al., 2004, 2005, 2007b, 2010; Duftner et al., 2005). Estimates without prior constraints on the MRCA of the Lamprologini revealed only slightly more recent age estimates for splits within the Lamprologini, but confidence intervals were largely overlapping with the results of the analysis in which we constrained the MRCA of the Lamprologini to an age of 5–6 MYA. Likewise, using a prior on the time window for a possible colonization of the Congo system via the Lukuga River had little effect on the resulting age estimates. The alternative calibrations, congruent with Genner et al.'s (2007) fossil calibrated and Gondwanan based scenarios for cichlid diversification yielded roughly two and five times older node ages, respectively (Table 2; Supplementary Figs. 3 and 4).

4. Discussion

Perhaps the most intriguing question connected to the diversification of the Lamprologini is the role of sexual selection. While this is considered highly important in mouthbrooding cichlid lineages (Dominey, 1984; Seehausen, 2000; Terai et al., 2006; Seehausen et al., 2008; Salzburger, 2009), substrate-breeding species with pair-formation lack many features associated with sexual selection as a diversifying force. Unlike haplochromines and several other mouthbrooding cichlid tribes, lamprologine cichlids never display pronounced sexual dichromatism upon which sexual selection via mate choice might act (Schluter and Price, 1993; Gray and McKinnon, 2007), indicating that color is not their basis for mate choice. However, while mating color can be ruled out as an important factor facilitating "proper" mate choice and driving reproductive isolation and speciation in this group, this may not be the case for other male-specific traits such as larger body size and territory quality and size. In fact, sexual size dimorphism is quite frequently observed in lamprologines, with males typically slightly larger than females, so that positive selection on male body size facilitating territorial defense seems likely (see e.g. Schütz et al., 2006; Maan and Taborsky, 2008). However, the most pronounced cases of sexual size dimorphism are observed in some obligatory and facultative gastropod shell breeders (*L. callipterus*, *L. lemairii*, *L. ornatipinnis*, *N. calliurus*, *N. fasciatus*). In *L. callipterus*, *L. lemairii* (some females appear to reach maturity at a body size small enough to spawn in gastropod shells) and *N. fasciatus*, females are small en-

ough to fit in a *Neothauma* shell, while males are too large to enter the shells and thus have to release their sperm over the shell entrance. In *L. callipterus* an alterative reproductive tactic evolved, with dwarf males that are even smaller than females and thus able to enter the shells to fertilize the eggs, even in the presence of the large territorial male. The point may be that male color traits can be pushed by diversifying sexual selection which is not the case for male fitness traits, so that selection acting on those cannot easily lead to two novel species in sympatry. However, divergent selection acting on polymorphic and naturally selected traits relevant for differential trophic specialization, as described for Central American cichlids (Barluenga and Meyer, 2004), may as well have promoted (sympatric) speciation in lamprologines.

Sexual selection can also act in social context, to produce signals for intraspecific communication. This is the case in lamprologines with brood-care helper behavior which often show individual color markings on the head or body. The *N. pulcher* group and perhaps the genus *Julidochromis* may serve as examples. The subclade comprising the *N. pulcher* group with species exhibiting sophisticated brood-care helping behavior is monophyletic according to our AFLP phylogeny (Fig. 4) and species-rich. All species display distinct color patterns on the head which are species-specific, albeit variable enough that individuals can be distinguished (Hert, 1985; Balshine-Earn and Lotem, 1998; Frostman and Sherman, 2004). It thus seems likely that the "head mask" facilitates social interactions and is thus subject to sexual selection. As most of these species are allopatric, maintenance and evolution of color in this group may be similar to the pattern found in the maternal mouthbrooding genus *Tropheus*, for which about 120 distinctly colored populations or sister species were described. As in the *N. pulcher* group, no sexual color dimorphism exists in *Tropheus*, and most color morphs are allopatric, so that divergent selection in sympatry seems unlikely as driving force of speciation (Egger et al., 2010). Taken together, disruptive sexual selection does not seem to be frequent in the Lamprologini, albeit other diversifying selective processes in social context might exist, in addition to those in the context of natural selection (see also Verburg and Bills, 2007; Takahashi et al., 2009). These might help to explain why the Lamprologini are the only non-mouthbrooding cichlid lineage which has formed a species flock with substantial diversity in sympatry.

Several lamprologine species show various degrees of population genetic structure and/or geographic variation in morphology (e.g. Duftner et al., 2006; Koblmüller et al., 2007c; Risch and Snoeks, 2008; Nevado et al., 2009) or form geographic sister species (Duftner et al., 2007), pointing to the influence of ecological specialization on the dispersal ability promoting allopatric speciation. Apparent correlation between habitat specialization and dispersal ability and degree of genetic structure and species richness has been inferred not only for lamprologines but other Lake Tanganyika cichlids as well (Taylor et al., 2001; Stiver et al., 2004; Duftner et al., 2006, 2007; Koblmüller et al., 2007c; Koblmüller et al., 2009; Sefc et al., 2007; Nevado et al., 2009; Takahashi et al., 2009; Wagner and McCune, 2009). Another peculiarity of the lamprologine cichlids is the high frequency of interspecific gene flow for a variety of reasons discussed below. Hybridization is also likely to be involved in speciation in particular lamprologine lineages (Salzburger et al., 2002a; Schelly et al., 2006; Koblmüller et al., 2007a), contributing to the species richness of this tribe. Remarkably, on the other hand, it seems that the hybridization events in the history of several species did not wipe out the involved species.

4.1. Phylogenetic implications

Our re-analysis is fully compatible with previous works (Sturmbauer et al., 1994; Schelly et al., 2006; Day et al., 2007; Koblmüller et al., 2007a), but adds several new groupings. Considering that the inconsistent placement of *N. buescheri* in the mitochondrial MP tree was most likely caused by long-branch-attraction (Hendy and Penny, 1989), the 79 species analyzed were grouped into 12 clades (named clades I–XII in this study) which can be further grouped into 6 major mtDNA lineages, as clades VI–XII are likely to be monophyletic. Just as in previous analyses, the Congo River lamprologines were not recovered as sister clade to the lake endemics. Strikingly, *N. devosi* from the Malagarazi River was most closely related to a lake endemic (*T. temporalis*), although it was grouped in the same subclade of clade XII as its Congo River allies, together with *N. petricola* and *N.* sp. "eseki". Thus, the inclusion of the Malagarazi-lamprologine corroborates our hypothesis that the most ancestral lineages of the tribe Lamprologini lived in the lake proper (Sturmbauer et al., 1994) and that all riverine species diverged from their lake allies about 1.7 and 1.2 MYA. The possibility that the most ancient branch of the Lamprologini lived in the Proto-Malagarazi-Congo River and seeded the lacustrine radiation can be ruled out. Instead, lamprologines apparently colonized the Congo system via the Lukuga River, Lake Tanganyika's only outflow, in the time window of 1.1–3.5 MYA during which this connection between Lake Tanganyika and the Congo system was open (Lezzar et al., 1996; Cohen et al., 1997). In our new phylogeny, para- or polyphyly of the genera *Lamprologus*, *Neolamprologus*, *Julidochromis*, *Chalinochromis* and *Telmatochromis* is evident, but most likely for different reasons which are discussed below. We should note that we consider the genus *Lepidiolamprologus* as revised according to Schelly et al. (2006).

Concerning the mtDNA – based groupings, clade I comprises two subclades, the first including *N. mustax*, *N. nigriventris*, *N. cylindricus* and *Neolamprologus bifasciatus*. Its second subclade – except for *N. niger* – exclusively comprises species with complex social system in which elder offspring contributes to brood care and territorial defense (Kuwamura, 1997). These species are *N. gracilis*, *N. savoryi*, *N. olivaceous* and *N. marunguensis*. In the mtDNA phylogeny three of them (*N. gracilis*, *N. savoryi* and *N. marunguensis*) are para- or polyphyletic with respect to their clade assignment, in that additional individuals were grouped in a subclade of clade XII, as previously shown for *N. marunguensis* (Salzburger et al., 2002b). Recent reciprocal gene flow, at least in one ancestral species, is further substantiated by this new evidence. The nuclear multilocus tree based on AFLP suggests monophyly of these taxa in the hypothetical species tree, with additional information from the mtDNA tree suggesting a past hybridization or introgression event twisting the tree, or less likely ancient incomplete lineage sorting. Clade II was already defined by Sturmbauer et al. (1994) as a highly diverse species group adapted to a variety of truly lacustrine ecological niches and named the "ossified group" on morphological grounds by Stiassny (1997). This clade also contains the predatory genus *Lepidiolamprologus*, which was recently revised by Schelly et al. (2006). The ossified group – lamprologines comprise perhaps the greatest ecological and behavioral diversity in that they contain both the largest predatory *Lepidiolamprologus* and the smallest cichlid species breeding in gastropod shells. Here we note great taxonomic incongruence within certain gastropod shell breeders, namely the sister species pair *N. multifasciatus* and *N. similis*, and the *L. ocellatus* species-assemblage (*L. ocellatus*, *L. meleagris* and *L. speciosus*), which on morphological and behavioral grounds must be grouped together. This issue is treated in detail in a separate paper, so that we briefly refer to our hypothesis of repeated hybridization events which was discussed elsewhere (Koblmüller et al., 2007a). Clade III represents a new lineage formed by two eco-morphologically similar species, *N. sexfasciatus* and *N. tretocephalus*. These genetically close sister taxa are the only descendents of an ancient lineage. Likewise, clade IV comprises *N. toae* only as monotypic representative of another ancient lineage. The same applies

to clade V which comprises *V. moorii*. This taxon was suggested as the most ancestral split in the pioneering molecular work of Sturmbauer et al. (1994), but now allies with 5 other ancient lineages. Clade VI was also identified earlier to comprise *N. longior*, and is now extended to also contain *N. leleupi*, *N. prochilus* and *N. obscurus*. Clade VII again is monotypic comprising *N. buescheri* only. As discussed above, this species jumps inside clade I in MP, most likely due to long-branch attraction (Hendy and Penny, 1989). Clade VIII comprises *N. cunningtoni* (formerly *Lepidiolamprologus*; see Schelly et al., 2006), sister to *N. modestus* and *N. tetracanthus*. Clade IX comprises *N. christyi* only. We note that a highly similar species, tentatively named *Neolamprologus* sp. "eseki" is grouped in clade XII. Clade X is also monotypic with *N. furcifer* only. Clade XI comprises three species of the genus *Chalinochromis* plus *J. ornatus*, *J. transcriptus* and *J. dickfeldi*, but excluding *J. marlieri* and *J. regani* which are grouped in clade XII. Finally, clade XII comprises a highly complex and eco-morphologically as well as behaviorally heterogeneous array of taxa. The first subclade (clade XII.1) comprises *N. falcicula* only, the second is comprised by *N. petricola*, the two Congo River lamprologines *L. congoensis* and *L. teugelsi*, sister to (*T. vittatus* (*N.* sp. "eseki" (*N. devosi* (*T. temporalis*)))). It should be noted that one additional haplotype of *T. temporalis* is placed in paraphyly.

Concerning the AFLP tree, it turned out that it was surprisingly compatible with the mtDNA phylogeny, corroborating 7 of the 12 clades with good bootstrap support. The remaining 5 clades were subdivided. As in the mtDNA phylogeny, most branches between the clades were short, corroborating that their diversification was rapid and established early in the lamprologine radiation. However, the AFLP data were able to shed light on several problematic placements in the mtDNA phylogeny: Contrasting with the mtDNA tree, all species which were placed para- or polyphyletically by mtDNA resulted as monophyletic in the AFLP tree. The genus *Telmatochromis*, which was scattered almost all over the mtDNA tree, turned out as monophyletic in the AFLP analysis, likewise the *Neolamprologus* species with brood-care helper behavior within clade XII.3. Interestingly, the paraphyletic placement of the genus *Julidochromis* was retained, corroborating the close relationship of *J. ornatus*, *J. transcriptus* and *J. dickfeldi* with the genus *Chalinochromis* and a somewhat more distant relationship to *J. marlieri* and *J. regani*. This paraphyly is most likely not due to ancient incomplete lineage sorting, given the large number of partially well supported nodes between the two *Julidochromis* clades (McCracken and Sorensen, 2005), but rather indicates convergent evolution of the particular *Julidochromis* phenotype with its characteristic body coloration. Also, the form of the genital papilla that clearly differs between the representatives of the two clades rejects a rather close relationship. The genital papilla of *Chalinochromis* on the other hand is very similar to that of *J. dickfeldi*, *J. ornatus* and *J. transcriptus*, further supporting the inferred phylogenetic relationships. Another aspect might be an ancient hybridization event among ancestral species of these two lineages, which might explain their partial morphological similarity despite long separate evolution afterward (Salzburger et al., 2002b).

4.2. Para- or polyphyletic taxa

While in some genera para- or polyphyly seems to be most likely the result of ancient ancestral polymorphism, it may be due to introgressive hybridization in others. A third alternative may be inadequate taxonomic assignment due to lack of diagnostic synapomorphic characters, causing genera defined by plesiomorphic traits. Previous studies (Sturmbauer et al., 1994; Stiassny, 1997; Schelly et al., 2006) diagnosed such taxonomic problems for the genera *Lamprologus*, *Neolamprologus* and *Lepidiolamprologus*. Our study confirms these problems and adds the genera *Julidochromis* and *Chalinochromis* to the list. Two cases of strong incompatibility of the mtDNA phylogeny with taxonomic assignments were found in the brood-care helper species *N. marunguensis* with its behavioral and morphological allies and the predatory species *L. nkambae* with its sister species *L. elongatus* and *L. kendalli*. As these taxa were resolved with their allies in the nuclear DNA tree, ancient incomplete lineage sorting or introgressive hybridization was taken into account, and both studies opted in favor of introgressive hybridization as the more likely reason (Salzburger et al., 2002b; Schelly et al., 2006). In many cases, however, it is impossible to rule out one of the two options, as both scenarios are realistic and might even be involved during the diversification of a lineage.

We found more problematic placements in the mtDNA phylogenies suggesting para- or polyphyly of species, similar to that observed in *N. marunguensis* (Salzburger et al., 2002b): These concerned *N. savoryi*, *N. gracilis*, *T. temporalis* and *T. dhonti*, as well as a case of complete mtDNA exchange in all *L. callipterus* of the northern basin of Lake Tanganyika after introgression of *N. fasciatus* mtDNA (Nevado et al., 2009). While it seems possible to argue in favor of ancient incomplete lineage sorting of an ancestral polymorphism in the cases of generic para- or polyphyly (as seen in *Telmatochromis* and *Julidochromis*), introgression and subsequent mtDNA capture seems a more likely explanation for the paraphyly within species (as seen in *N. marunguensis*, *N. savoryi*, and *N. gracilis*; see also Salzburger et al., 2002b; Schelly et al., 2006 and Nevado et al., 2009 for more detailed arguments). The additional cases presented here of reticulation among taxa with brood-care helper behavior spread over clades I and XII highlight once more that introgressive hybridization is more frequent in the lamprologines than in any other tribe studied.

Considering the high frequency of trans-specific gene flow one must ask for possible reasons. Unlike all other Tanganyikan lineages except for *B. microlepis* they are substrate breeders forming stable pairs or harems. Unlike riverine substrate breeders of the Tilapiine or Pelvicachromine lineages, several species display complex social behavior. Moreover, several social species form arrays of allopatric sister species which most likely evolved in allopatry. These species seem to remain reproductively coherent for long time spans, so that lake-level fluctuations may connect previously disjunct species and cause introgression or hybridization as discussed in detail in Salzburger et al. (2002b). The following reticulations are suggested from the closest sister group relationships within and among the two mtDNA clades: *N. savoryi* with *N. marunguensis*; *N. savoryi* and *T. temporalis*; *N. savoryi* and *T. dhonti*; *N. savoryi* and *T. bifrenatus*; *N. gracilis* and *N. marunguensis* and/or *N. savoryi*. These cases have in common that the close relatedness of the introgressed mtDNA genotypes to populations or sister species of the donor species argues against ancestral polymorphism. A second array of lamprologine species living in close connection to each other concerns gastropod shell dwellers. They often live and spawn in extremely close vicinity, e.g. in *L. callipterus* nests, so that they are at higher risk for accidental cross-species fertilizations. That such hybridization cases are extremely rare can be ruled out, as (F1-) hybrids were repeatedly observed in *L. callipterus* nests (Aibara, pers. comm., Koblmüller et al., 2007a) as were heterospecific breeding pairs in other lamprologine species (Fig. 6). We thus suggest that substrate breeders with complex social behavior, as adaptation to a life in densly packed species communities, may be more susceptible to trans-specific gene flow than maternal mouthbrooders.

Concerning the third alternative, para- or polyphyly of species due to inadequate taxon sampling or lack of synapomorphic characters, two reasons can be put forward. The first would involve taxonomic error to be corrected in a revision including molecular phylogenies and additional synapomorphic characters, whatever

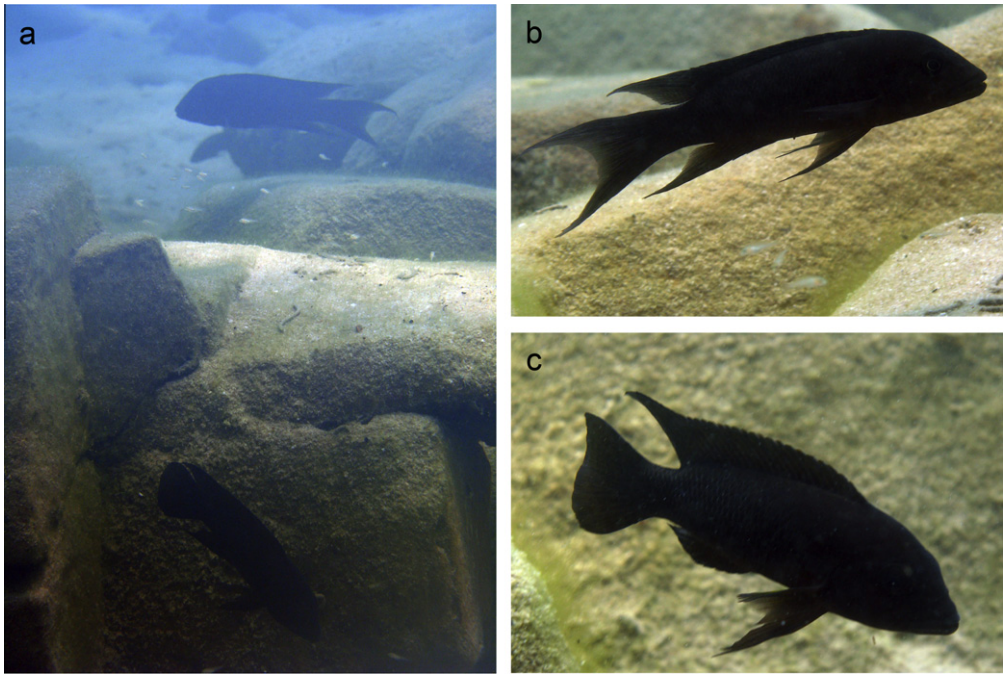

Fig. 6. Hetero-specific breeding pair (*Neolamprologus christyi* × *N. modestus*) guarding its fry near the Kalambo estuary in the southeast of Lake Tanganyika; (a) the pair at the breeding site; and the two parents (b) *N. christyi* and (c) *N. modestus*. The most obvious difference between the two species is the shape of the caudal fin.

traits they might be. The second reason is a much more intriguing one, in that evolution will necessarily produce para- or even polyphyletic taxa, when new species evolve strikingly novel features within a homogeneous clade, as exemplified by the evolution of birds within a clade of dinosaurs. In analogy, the genera *Lamprologus* or *Neolamprologus* may as well be considered as a plesiomorphic *bauplan* from which multiple sub-lineages with novel traits branched off. Given the scenario of multifold subdivision of rocky shores by ecological barriers, local innovation and speciation should prevail in the Lamprologini, as e.g. in the Tropheini for which parallel evolution of genus-diagnostic dentition types was also suggested (Sturmbauer et al., 2003; Koblmüller et al., 2010). Molecular phylogenies will be useful to trace the ancestral states of various clades, to identify the ancestral generic features.

An interesting point for discussion is the validity of paraphyletic genera. Should it turn out that the morphological features defining the genus *Neolamprologus* were originally apomorphic traits to be repeatedly modified in various sub-lineages by further innovation, one could argue to retain this genus name for all now plesiomorphic species and revise all genera with novel traits in monophyletic genera defined by certain apomorphic features. In this way necessary taxonomic adjustments can be carried out, but a "novel genus inflation" avoided.

4.3. Timing and pace of the lamprologine radiation

Age estimates based on molecular phylogenetic trees are based on a series of assumptions and require that four general conditions be met: (i) an accurate and well supported tree that resolves the important nodes in the phylogeny; (ii) reliable calibration points that provide upper and lower bounds for the nodes of interest; (iii) molecular clock methods that account for substitution rate heterogeneity within and across lineages; and (iv) a broad taxon sampling that includes the entire diversity in lineages (Soltis et al., 2002). Unfortunately, reliable calibration points for estimating divergence times in the East African cichlid species flocks are scarce, with no fossils available from these lacustrine species flocks. Thus, estimation of divergence times in East African cichlids has mainly relied on the assumption that the onset of these spectacular intralacustrine radiations coincided with the formation of a real lacustrine habitat in Lake Tanganyika and the refilling of Lakes Malawi and Victoria following their desiccation (Cohen et al., 1993, 1997; Delvaux, 1995; Johnson et al., 1996; Lezzar et al., 1996; Stager and Johnson, 2008). Only recently, alternative age estimates for the East African cichlid species flocks have been proposed based on independent external calibration points (Genner et al., 2007; Schwarzer et al., 2009) resulting in considerably older divergence time estimates. However, these recent developments are not free of pitfalls and in particular difficult to reconcile with the biologic characteristics of the Lake Tanganyika cichlid species flock, such that these calibration methods are quite problematic (for a detailed discussion see Koblmüller et al., 2008a, b). The chronograms based on these alternative calibrations are shown in Supplementary Figs. 3 and 4. We decided to use the following calibration points (for justification see Koblmüller et al., 2008a, b): The establishment of a real lacustrine habitat in Lake Tanganyika with clear- and deep-water conditions 5–6 MYA (Tiercelin and Mondeguer, 1991; see also Salzburger et al., 2002), the time window for the Congo River *Lamprologus* to leave the lake via the Lukuga (Lezzar et al., 1996; Cohen et al., 1997), and the age of the Lake Malawi species flock (Delvaux, 1995; Sturmbauer et al., 2001). There are ongoing analyses of a sediment core of the Lake Malawi deep basin at a depth of 650 m, which will without doubt result in a more precise estimate for the onset of lacustrine conditions in Lake Malawi after a dryup or near-dryup (Andrew Cohen, personal communication).

Contrasting the general diversification patterns observed in the mouthbrooding Lake Tanganyika cichlid tribes (Salzburger et al., 2002a; Sturmbauer et al., 2003; Brandstätter et al., 2005; Koblmüller et al., 2004, 2005, 2007b, 2010; Duftner et al., 2005), the Lamprologini diversified more gradually with no clear bursts of speciation evident (also see Day et al., 2008), albeit a concentration of cladogenetic events about 2.5–3 MYA coincided with a period of increased diversification rates in the mouthbrooding cichlid lineages. Many species seem to be rather old, whereas recent specia-

tion (<1 MYA) predominantly occurred among the brood-care helper cichlids, in the *Julidochromis* assemblage and (mainly) the shell-breeding members of the genus *Lepidiolamprologus*. The ecological and behavioral characteristics of these species point to a reduced dispersal capacity, implying high degrees of population structure, as has already been demonstrated for the *N. brichardi*/*N. pulcher* complex (Stiver et al., 2004; Duftner et al., 2007) and an increased potential for allopatric speciation. Indeed, most members of these groups are allopatrically distributed and some occur only along very short stretches of shoreline, indicating that ecological specialization resulted in increased diversification rate in these groups.

4.4. Taxonomic implications

A revision of the species and genera of the Lamprologini is needed and we hope to contribute valuable information for future assignments. Clearly, this work needs to be based on the original descriptions of Boulenger (1898) and Max Poll's insightful revisions (1956, 1986), as well as on more recent work of others (Stiassny, 1991, 1997; Colombe and Allgayer, 1985; Takahashi, 2003; Schelly and Stiassny, 2004; Schelly et al., 2006). As discussed by Stiassny (1992), meristic and morphometric measurements, osteology and dentition alone will not suffice to achieve the goal, as too many characters are homoplastic. Concerning the genus *Neolamprologus*, its description was based on the assumption that the Congo River species branched ancestrally to the rest, so that *Lamprologus* was retained in those species only, except for *L. callipterus*. A viable way might be to re-assign the genus name *Lamprologus* to most *Neolamprologus* species. As *L. congoensis* is the type species of the genus *Lamprologus*, this would be a viable scenario. The type species of the genus *Neolamrologus* is *N. tetracanthus*. Thus, when keeping the genus *Neolamprologus*, priority must be given to the clade also containing *N. tetracanthus* (clade VIII), also including the species *N. modestus* and *N. cunningtoni*, (plus *N. christy*, *N. mondabu* and *N. petricola* on the basis of nuclear DNA data). Naming all members of the ossified group a new genus name *Stiassnia* as recently suggested by Day et al. (2007), will not solve the problem, but cause further confusion, as perfectly valid genera such as *Lepidiolamprologus* (Schelly et al., 2006, 2007) and *Altolamprologus* (but excluding *N. fasciatus*; contrasting Day et al., 2007; see also Koblmüller et al., 2007a, and Nevado et al., 2009) would be subsumed, neglecting the huge eco-morphological and behavioral diversity of the ossified group.

Considering molecular traits and brood-care helping behavior as synapomorphic traits, the *N. brichardi* assemblage including *N. pulcher*, *N. helianthus*, *N. marunguensis*, *N. olivaceous* and *N. sarvori*, may be a good candidate group for a new genus, as would be the clade comprising *N. tetracanthus*, *N. cunningtoni*, *N. christyi*, *N. petricola*, *N. modestus* and *N. mondabu*. Concerning *Julidochromis*, a split in one group retaining the genus name (*J. marlieri* and *J. regani*), and a new genus comprising all *Chalinochromis* and *J. ornatus* and *J. dickfeldi* is suggested by molecular data and the shape of the male genital papilla. This could be achieved by revising the latter species as *Chalinochromis*.

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Appendix A

List of samples used for mtDNA and AFLP analyses, with sampling locality and coordinates (if known) of lamprologine taxa and GenBank accession numbers for the ND2 gene.

Species	SampleID	Sampling locality	Coordinates	GenBank Acc. No. ND2	AFLPs
Altolamprologus calvus	2481	Nakaku	S 8°40', E 30°54'	EF191108	–
	3899	Kapembwa	S 8°37', E 30°51'	-	+
	4099	Chaitika	S 8°34', E 30°47'	-	+
Altolamprologus compressiceps	2482	Nakaku	S 8°40', E 30°54'	EF191105	–
	3900	Kalambo Lodge	S 8°37', E 31°37'	-	+
	3988	Kalambo	S 8°36', E 31°11'	-	+
Altolamprologus sp. "shell"	0586	Sumbu	S 8°31', E 30°29'	EF191107	–
Chalinochromis brichardi	3463	Ulwile	S 7°27', E 30°34'	HM623820	
	5608	Kalambo Lodge	S 8°37', E 31°37'	-	+
	5708	Kalambo Lodge	S 8°37', E 31°37'	-	+
Chalinochromis popelini		Aquarium trade		U07244	–
Chalinochromis sp. "kipili"	0295	Aquarium trade		HM623802	–
Chalinochromis sp. "ndobnoi"	0294	Aquarium trade		HM623801	–
Julidochromis dickfeldi	0591	?		HM623790	–
	5616	?		-	+
	5733	?		-	+
Julidochromis marlieri	2055	Kalambo	S 8°36', E 31°11'	HM623819	–
	5663	?		-	+

(*continued on next page*)

Appendix A (*continued*)

Species	SampleID	Sampling locality	Coordinates	GenBank Acc. No. ND2	AFLPs
Julidochromis ornatus	0880	Nkondwe Island	S 7°23', E 30°33'	HM623791	–
	2192	Kasakalawe	S 8°47', E 31°05'	EF191082	–
	4350	Kasakalawe	S 8°47', E 31°05'	-	+
Julidochromis regani	3461	Masaka	S 5°02', E 29°46'	HM623818	–
	5667	Kaseke		-	+
	5680	Kaseke		-	+
Julidochromis transcriptus	1222	?		HM623792	–
	5599	Gombe		-	+
Lamprologus callipterus	2517	Wonzye	S 8°43', E 31°08'	EF191085	–
	4263	Wonzye	S 8°43', E 31°08'	-	+
	4270	Wonzye	S 8°43', E 31°08'	-	+
Lamprologus congoensis	0307	?		AY740385	–
Lamprologus kungweensis	2521	Aquarium trade		EF191084	–
Lamprologus laparogramma	0579	Mpulungu	S 8°46', E 31°06'	EF191087	–
Lamprologus lemairii	0582	Mpulungu	S 8°46', E 31°06'	AY740386	–
Lamprologus meleagris	1733	Aquarium trade		DQ055027	–
Lamprologus ocellatus	1833	Chisansa	S 8°39', E 31°11'	EF191113	–
Lamprologus cf. ocellatus[*]	2518	Aquarium trade		EF191116	–
Lamprologus ornatipinnis	1904	Aquarium trade		EF191112	–
Lamprologus signatus	0578	Mpulungu	S 8°46', E 31°06'	EF191086	–
Lamprologus speciosus	1908	Aquarium trade		EF191102	–
Lamprologus teugelsi	2519	Aquarium trade		HM623815	–
	5658	Aquarium trade		-	+
	5677	Aquarium trade		-	+
Lepidiolamprologus attenuatus	1828	Mpulungu	S 8°46', E 31°06'	DQ055036	–
	3906	Mtondwe Island	S 8°42', E 31°07'	-	+
Lepidiolamprologus boulengeri	1909	Aquarium trade		DQ055040	–
Lepidiolamprologus elongatus	3644	Kalambo Lodge	S 8°37', E 31°37'	HM623829	–
	3909	Katoto	S 8°48', E 31°01'	-	+
Lepidiolamprologus hecqui	1913	Aquarium trade		DQ055041	–
Lepidiolamprologus kendalli	0355	Aquarium trade		DQ055060	–
Lepidiolamprologus meeli	2479	Kigoma	S 4°52', E 29°37'	DQ055051	–
Lepidiolamprologus nkambae	1752	Aquarium trade		DQ055035	–
Lepidiolamprologus profundicola	3652	Kasenga Rocks	S 8°43', E 31°09'	HM623830	–
	4107	Kasenga Rocks	S 8°43', E 31°09'	-	+
Lepidiolamprologus sp. "meeli-boulengeri"	1831	Mbita Island	S 8°48', E 31°01'	DQ055038	–
	3915	Muzumwa	S 8°42', E 31°12'	-	+
Lepidiolamprologus variostigma	1738	?		DQ055029	–
Neolamprologus bifasciatus	1820	Aquarium trade		HM623809	–
Neolamprologus brevis	1730	Aquarium trade		EF191095	–
Neolamprologus brichardi	1479	?		DQ05515	–
	5682	Fulwe Rocks		-	+
	5683	Fulwe Rocks		-	+
Neolamprologus buescheri	1745	Tembwe	S 7°14', E 30°07'	HM623803	–
	5729	?		-	+
	5739	?		-	+
Neolamprologus calliurus	2522	Aquarium trade		EF191117	–
Neolamprologus caudopunctatus	0208	Aquarium trade		AY740388	–
Neolamprologus christyi	3622	Isanga	S 8°39', E 31°12'	HM623826	–
	5617	Isanga	S 8°39', E 31°12'	-	+
	5664	Isanga	S 8°39', E 31°12'	-	+
	5676	Isanga	S 8°39', E 31°12'	-	+
Neolamprologus cunningtoni	2513	Kapata		DQ055053	–
	5695	Wonzye	S 8°43', E 31°08'	-	+
	5732	Kalambo Lodge	S 8°37', E 31°37'	-	+
Neolamprologus cylindricus	3618	Aquarium trade		HM623823	–
	5657	?		-	+
Neolamprologus devosi	1819	Ivagala (Malagarazi)		EF437476	–
Neolamprologus falcicula	3436	Aquarium trade		HM623817	–
Neolamprologus fasciatus	2516	Wonzye	S 8°43', E 31°08'	EF191120	–
	4233	Wonzye	S 8°43', E 31°08'	-	+

Appendix A (*continued*)

Species	SampleID	Sampling locality	Coordinates	GenBank Acc. No. ND2	AFLPs
	4249	Wonzye	S 8°43', E 31°08'	-	+
Neolamprologus furcifer	2126	?		HM623812	–
	5734	?		-	+
Neolamprologus gracilis	0625	Kalo		HM623798	–
	3434	Kigoma	S 4°52', E 29°37'	HM623816	–
Neolamprologus helianthus	1477	?		DQ055013	–
	5668	Aquarium trade		-	+
	5674	Aquarium trade		-	+
Neolamprologus leleupi	2147	Aquarium trade		HM623814	–
	5660	Aquarium trade		-	+
	5661	Aquarium trade		-	+
Neolamprologus leloupi	1906	Aquarium trade		EF191103	–
	1907	Aquarium trade		EF191104	–
Neolamprologus longior	0216	Aquarium trade		HM623793	–
Neolamprologus marunguensis	0618	Kafitilila		AY740390	–
	0619	Kafitilila		HM623797	–
	5718	Aquarium trade		-	+
	5719	Aquarium trade		-	+
Neolamprologus modestus	3457	Kasakalawe	S 8°47', E 31°05'	HM623821	–
	5596	Kalambo Lodge	S 8°37', E 31°37'	-	+
	5597	Kalambo Lodge	S 8°37', E 31°37'	-	+
Neolamprologus mondabu	3433	Mpimbwe	S 7°08', E 30°30'	-	+
Neolamprologus multifasciatus	0310	Aquarium trade		EF191089	–
	3nmulti	Aquarium trade		-	+
	5662	Aquarium trade		-	+
Neolamprologus mustax	2118	Mtondwe Island	S 8°42', E 31°07'	HM623811	–
	5704	Mbita Island	S 8°48', E 31°01'	-	+
	5659	Kapembwa	S 8°37', E 30°51'	-	+
Neolamprologus niger	0315	?		AY740391	–
Neolamprologus nigriventris	1227	?		AY740392	–
Neolamprologus obscurus	2946	Kasakalawe	S 8°47', E 31°05'	HM623824	–
	5741	Kasakalawe	S 8°47', E 31°05'	-	+
	5742	Kasakalawe	S 8°47', E 31°05'	-	+
Neolamprologus olivaceous	0631	Kyeso	S 6°30', E 29°29'	AY740393	–
	5656	Aquarium trade		-	+
Neolamprologus petricola	3631	Kapembwa	S 8°37', E 30°51'	HM623827	–
	5675	S of Katoto	S 8°48', E 31°01'	-	+
Neolamprologus prochilus	3623	Kalambo	S 8°36', E 31°11'	HM623825	–
	5582	Kalambo	S 8°36', E 31°11'	-	+
Neolamprologus pulcher	521	Mbita Island	S 8°48', E 31°01'	HM623795	–
	5601	Katukula	S 8°43', E 30°57'	-	+
	5684	Mbita Island	S 8°48', E 31°01'	-	+
Neolamprologus savoryi	613	Kyeso	S 6°30', E 29°29'	HM623807	–
	614	Kimomo		HM623796	–
	1463	Kachese	S 8°29', E 30°28'	HM623800	–
	1511	Kachese	S 8°29', E 30°28'	HM623810	–
	2145	S of Loasi River	S 8°19', E 31°04'	HM623805	–
	2146	Mbita Island	S 8°48', E 31°01'	HM623806	–
	5752	?		-	+
	5754	?		-	+
Neolamprologus sexfasciatus	3624	Kalambo	S 8°36', E 31°11'	HM623828	–
	5612	Katukula	S 8°43', E 30°57'	-	+
	5614	S of Katoto	S 8°48', E 31°01'	-	+
Neolamprologus similis	1739	Tembwe	S 7°14', E 20°07'	DQ055030	–
	1902	?		EF191100	–
Neolamprologus sp. "eseki"	0347	?		HM623794	–
Neolamprologus splendens	628	Kasu		HM623799	–
Neolamprologus tetracanthus	3458	Mpulungu	S 8°46', E 31°06'	HM623822	–
	5611	Kalambo Lodge	S 8°37', E 31°37'	-	+
	5697	Kalambo Lodge	S 8°37', E 31°37'	-	+
Neolamprologus toae	0217	Aquarium trade		AY682543	–

(*continued on next page*)

Appendix A (*continued*)

Species	SampleID	Sampling locality	Coordinates	GenBank Acc. No. ND2	AFLPs
	2148	Aquarium trade		HM623813	–
	5693	Aquarium trade		-	+
	5694	Aquarium trade		-	+
Neolamprologus tretocephalus	1731	Aquarium trade		DQ055026	–
	5649	Aquarium trade		-	+
	5650	Aquarium trade		-	+
Neolamprologus walteri	1816	near Kigoma (hilltop)	S 4°53', E 29°36'	HM623808	–
Telmatochromis bifrenatus	0106	Aquarium trade		DQ055009	–
Telmatochromis dhonti	1829	Mbita Island	S 8°48', E 31°01'	HM623787	–
	1830	Mtondwe Island	S 8°42', E 31°07'	HM623804	–
	5651	?		-	+
	5652	?		-	+
Telmatochromis temporalis	1475	Tongwa	S 8°40', E 30°53'	HM623789	–
	2117	Mbita Island	S 8°48', E 31°01'	HM623788	–
	5717	Funda	S 8°46', E 30°59'	-	+
	5735	?		-	+
Telmatochromis vittatus	2119	Mtondwe Island	S 8°42', E 31°07'	AY682545	–
	5594	Isanga	S 8°39', E 31°12'	-	+
Variabilichromis moorii	1508	Kachese	S 8°29', E 30°28'	DQ055016	–
	5751	?		-	+
	5753	?		-	+
Outgroups					
Boulengerochromis microlepis				AF317229	
Bathybates leo	2226			AY663731	–
Hemibates stenosoma	2234			AY663719	–
Telotrematocara macrostoma	2483			AY663715	–
Ophthalmotilapia ventralis				U07257	–
Callochromis macrops	1854			AY337795	–
	5653			-	+
	5654			-	+
Cyphotilapia frontosa				U07247	–
Triglachromis otostigma	0103			AY337769	–
Limnochromis auritus	0059			AY337766	–
Paracyprichromis brieni				AF398223	–
Perissodus microlepis				AF317265	–
	3240			EF437483	+
	3470			EF437484	+
	3597			EF437485	+
Benthochromis tricoti	1480			EU753962	–
Ctenochromis horei	2524			EU753935	–
Petrochromis sp. "macrognathus rainbow"	8259			GQ995772	–
Tropheus moorii				AB018975	–
	5598			-	+
	5600			-	+
Astatotilapia calliptera				EU753934	–
Aulonocara sp.	6662			GQ995712	–
Labidochromis caeruleus	3055			AY740383	–
Melanochromis auratus				AY930063	–
Nimbochromis livingstoni	3057			EU753948	–
Nimbochromis venustus	3056			EU753947	–
Pseudotropheus tropheops	3058			AY740384	–
Eretmodus cyanostictus	1102			DQ055010	–
	5678			-	+
Spathodus erythrodon	0092			DQ055008	–
	5673			-	+
Spathodus marlieri	0058			HM623786	–
Tanganicodus irsacae	0066			DQ055007	–

Sequences not generated in the framework of this study were obtained from GenBank from following publications: Kocher et al. (1995); Klett and Meyer (2002); Salzburger et al. (2002a, 2005); Koblmüller et al. (2004, 2005, 2007a,b, 2008, 2010); Duftner et al. (2005); Schelly et al. (2006).

* In a previous publication (Koblmüller et al., 2007a), this sample has been erroneously identified as *Neolamprologus wauthioni* (see Büscher, 2007).

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.06.018.

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