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A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach

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ABSTRACT

The species-flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi and Tanganyika constitute the most diverse extant adaptive radiations in vertebrates. Lake Tanganyika, the oldest of the lakes, harbors the morphologically and genetically most diverse assemblage of cichlids and contains the highest number of endemic cichlid genera of all African lakes. Based on morphological grounds, the Tanganyikan cichlid species have been grouped into 12–16 distinct lineages, so-called tribes. While the monophyly of most of the tribes is well established, the phylogenetic relationships among the tribes remain largely elusive. Here, we present a new tribal level phylogenetic hypothesis for the cichlid fishes of Lake Tanganyika that is based on the so far largest set of nuclear markers and a total alignment length of close to 18 kb. Using next-generation amplicon sequencing with the 454 pyrosequencing technology, we compiled a dataset consisting of 42 nuclear loci in 45 East African cichlid species, which we subjected to maximum likelihood and Bayesian inference phylogenetic analyses. We analyzed the entire concatenated dataset and each marker individually, and performed a Bayesian concordance analysis and gene tree discordance tests. Overall, we find strong support for a position of the Oreochromini, Boulengerochromini, Bathybatini and Trematocarini outside of a clade combining the substrate spawning Lamprologini and the mouthbrooding tribes of the 'H-lineage', which are both strongly supported to be monophyletic. The Eretmodini are firmly placed within the 'H-lineage', as sister-group to the most species-rich tribe of cichlids, the Haplochromini. The phylogenetic relationships at the base of the 'H-lineage' received less support, which is likely due to high speciation rates in the early phase of the radiation. Discordance among gene trees and marker sets further suggests the occurrence of past hybridization and/or incomplete lineage sorting in the cichlid fishes of Lake Tanganyika.

1. Introduction

The species-flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi and Tanganyika (LT) represent the most species-rich adaptive radiations known in vertebrates (see e.g. Kocher, 2004; Salzburger, 2009; Seehausen, 2006). Several hundred of endemic cichlid species have evolved in each of these lakes in only the last few million to several thousand of years (see e.g. Genner et al., 2007; Kocher, 2004; Salzburger, 2009; Salzburger and Meyer, 2004; Snoeks, 2000; Turner et al., 2001; Verheyen et al., 2003). Because of their taxonomic diversity, their ecological and morphological disparity and the high proportion of endemism, East African cichlid fishes are a prime model system in evolutionary biology (reviewed in: Kocher, 2004; Salzburger, 2009; Santos and Salzburger, 2012).

With a maximum estimated age of 9–12 million years (my), LT is the oldest lake in Africa (Cohen et al., 1997; Salzburger et al., 2014) and contains the genetically, morphologically and ecologically most diverse group of cichlid fishes counting ca. 200 species in more than 50 genera (Koblmüller et al., 2008b; Salzburger et al., 2002a; Snoeks, 2000). Based on morphological grounds, Poll (1986) grouped the LT cichlid species into 12 tribes (a taxonomic rank between subfamily and genus): Bathybatini, Cyprichromini, Ectodini, Eretmodini, Haplochromini, Lamprologini, Limnochromini, Perissodini, Tilapiini, Trematocarini, Tropheini,

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and Tylochromini. Takahashi (2003) revised Poll's tribal assignment and suggested to (i) taking *Boulengerochromis microlepis* out of the Tilapiini into its own tribe, Boulengerochromini, leaving behind *Oreochromis tanganicae* as the only representative of the Tilapiini in LT; (ii) splitting the Limnochromini into Limnochromini *sensu stricto*, Benthochromini and Greenwoodochromini; (iii) establishing a separate tribe, Cyphotilapiini, for *Cyphotilapia frontosa* and *C. gibberosa*; (iv) moving '*Ctenochromis*' *benthicola* into its own tribe; and (v) putting the species of the Trematocarini into the Bathybatini. Only some of these revisions are backed up by molecular data, such as the establishment of the new tribes Benthochromini, Boulengerochromini, and Cyphotilapiini (Koblmüller et al., 2008b; Muschick et al., 2012; Salzburger et al., 2002a). *Greenwoodochromis*, on the other hand, is clearly nested within the Limnochromini in molecular phylogenies (Duftner et al., 2005; Muschick et al., 2012; Kirchberger et al., 2014), and should hence remain within the Limnochromini; the Trematocarini consistently form a separate lineage outside the Bathybatini (see e.g. Koblmüller et al., 2005; Muschick et al., 2012) and should remain in their own tribe (note that Koblmüller et al. (2008b) suggested splitting the Bathybatini into Bathybatini *sensu stricto* and Hemibatini); and '*Ctenochromis*' *benthicola* has recently been identified as member of the Cyphotilapiini (Muschick et al., 2012). Finally, the Tropheini were consistently found to be nested within the Haplochromini (Salzburger et al., 2005, 2002a; see also below) and should, hence, not be considered as separate tribe but as part of the Haplochromini.

Not all of the cichlid tribes occurring in LT are endemic to this lake, though, and four tribes show a distribution range that exceeds the LT basin by far. The Tylochromini have their center of divergence in West Africa (Stiassny, 1990), and the only LT species, *T. polylepis*, is likely to have invaded LT only recently (Koch et al., 2007). The same might be true for *O. tanganicae*, the only native representative of the widely distributed Tilapiini in LT (Klett and Meyer, 2002). Note that the Tilapiini were recently taxonomically revised and that the genus *Oreochromis* has been placed into a new tribe, namely the Oreochromini (Dunz and Schliewen, 2013). The Lamprologini, the most species-rich tribe of cichlids in LT, contain a few species that have secondarily colonized the Congo and Malagarasi River systems (Salzburger et al., 2002a; Schelly et al., 2003; Schelly and Stiassny, 2004; Sturmbauer et al., 2010). The Haplochromini (including the Tropheini) represent the most species-rich tribe of cichlids overall, and are distributed across large parts of Africa, where they have seeded various radiations including the ones of Lake Malawi and the Lake Victoria Region (Koblmüller et al., 2008a; Salzburger et al., 2005; Schwarzer et al., 2012; Verheyen et al., 2003; Wagner et al., 2012). The LT cichlid fishes thus show faunal affinities across a large geographical range to both older cichlid lineages such as the Tylochromini and Tilapiini/Oreochromini and younger ones such as the Haplochromini.

The phylogenetic relationships among East African cichlid tribes has been the subject of various studies over the past two decades, yet remain enigmatic (reviewed in: Koblmüller et al., 2008b). The first comprehensive phylogenetic study of LT's cichlid fishes using molecular information dates back to the early 1990s, when Nishida (1991) used allozyme data to examine the relationships among tribes. He established the so-called 'H-lineage' consisting of the tribes Cyprichromini, Ectodini, Eretmodini, Haplochromini/Tropheini (which he already found to be monophyletic), Limnochromini, and Perissodini as sister-group to the Lamprologini; the Bathybatini, Trematocarini plus *Boulengerochromis microlepis*, *Oreochromis tanganicae*, and *Tylochromis polylepis* were placed outside of a clade formed by the 'H-lineage' and Lamprologini. Yet, the relative position of the 'H-lineage' tribes differed depending on the algorithms used (UPGMA and neighbour-joining; NJ) (Fig. 1a). Sturmbauer and Meyer (1993) used two mitochondrial (mt) DNA markers (cytochrome *b* and control region) and suggested, based on phylogenetic analyses with NJ and maximum parsimony (MP), a sister-group relationship between the Cyprichromini and the Ectodini and between the Eretmodini and the Haplochromini (Fig. 1b). Kocher et al. (1995) established the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene as marker for phylogenetic analyses in cichlid fishes and provided the most inclusive phylogenetic hypothesis for LT cichlids so far. In their MP and NJ phylogenies, the Bathybatini, the Tylochromini, *B. microlepis* and *O. tanganicae* formed a clade, and the Eretmodini were placed outside the 'H-lineage', as sister-group to the Lamprologini (Fig. 1c). The Cyprichromini were resolved as the sister-group to all remaining 'H-lineage' taxa (i.e. without the Eretmodini). Using three mitochondrial markers (control region, cytochrome *b*, ND2) and NJ, MP and maximum-likelihood (ML) phylogenetic analyses, Salzburger et al. (2002a) confirmed the position of *B. microlepis*, the Bathybatini and the Trematocarini outside all other tribes occurring in Lake Tanganyika, with the exception of the Tylochromini, and the Eretmodini were placed as sister-group to the Lamprologini and the remaining 'H-lineage' tribes (Fig. 1d). Within the 'H-lineage', the Ectodini appeared as the sister to the remaining taxa. This study was also the first to establish phylogenetic affinities between the LT cichlid fishes and the riverine genus *Orthochromis* (not shown in Fig. 1d; see also Salzburger et al., 2005). Clabaut et al. (2005) combined sequences of the mitochondrial ND2 gene and the nuclear recombinase activating gene (*rag*) and applied ML and Bayesian inference (BI). They placed the Eretmodini as sister-group to the Lamprologini and established the 'C-lineage', i.e. the 'H-lineage' of Nishida (1991) but without the Eretmodini. Within this 'C-lineage', the Limnochromini plus *C. frontosa* appeared as the sister-group to the Perissodini, the Ectodini, the Cyprichromini and the Haplochromini (Fig. 1e). Day et al. (2008) provided one of the most comprehensive datasets to date (cytochrome *b*, ND2) including 157 taxa. Their ML and BI phylogenies supported the existence of the 'C-lineage' by placing the Eretmodini as sister-group to the Lamprologini. In their analyses, a clade formed by the Ectodini and Cyprichromini was placed as the sister-group of the remaining 'C-lineage' taxa (Fig. 1f). In the ML phylogeny of Muschick et al. (2012), who used the mitochondrial ND2 gene and two nuclear markers (*ednrb1*, *phpt1*), the Eretmodini were placed as sister group to the Lamprologini and the 'C-lineage', within which the Limnochromini appeared outside of all other included taxa (Fig. 1g). The study of Friedman et al. (2013), which was based on ten nuclear makers and did not focus specifically on the species of LT but on a larger cichlid phylogeny, revealed a clade formed by the Lamprologini, the Perissodini plus the Cyprichromini, and the Cyphotilapiini plus the Limnochromini as sister group to the Ectodini, the Eretmodini and the Haplochromini (Fig. 1h).

In summary, after more than 20 years of research, the composition of individual LT tribes has been well investigated, whereas the phylogenetic relationships among these cichlid tribes remain largely elusive. All studies performed so far revealed different results (Fig. 1), and the support values for many of the deeper nodes were consistently low. While there is consensus about the position of *T. polylepis*, *O. tanganicae*, the Bathybatini, Boulengerochromini and Trematocarini outside of the other tribes, the following main areas of uncertainty persist: (i) the relative position of the Bathybatini, Boulengerochromini and Trematocarini to each other; (ii) the placement of the Eretmodini, which were suggested as either being part of the 'H-lineage' and sister to the Haplochromini (Friedman et al., 2013; Nishida, 1991; Sturmbauer and Meyer, 1993), as sister-group to the Lamprologini (Clabaut et al., 2005; Day et al., 2008; Kocher et al., 1995), or as separate lineage outside the Lamprologini-'C-lineage' clade (Muschick et al., 2012; Salzburger et al.,