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A Microsatellite-Based Genetic Linkage Map of the Cichlid Fish, *Astatotilapia burtoni* (Teleostei): A Comparison of Genomic Architectures Among Rapidly Speciating Cichlids

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ABSTRACT

Cichlid fishes compose an astonishingly large number of species and formed species flocks in record-breaking time. To facilitate efficient genome scans and comparisons of cichlid genomes, we constructed a medium-density genetic linkage map of microsatellite markers of *Astatotilapia burtoni*. The mapping cross was derived from two inbred laboratory lines to obtain F_2 progeny by intercrossing. The map revealed 25 linkage groups spanning 1249.3 cM of the genome (size ~950 Mb) with an average marker spacing of 6.12 cM. The seven *Hox* clusters, *ParaHox C1*, and two paralogs of *Pdgfr*β were mapped to different linkage groups, thus supporting the hypothesis of a teleost-specific genome duplication. The *A. burtoni* linkage map was compared to the other two available maps for cichlids using shared markers that showed conservation and synteny among East African cichlid genomes. Interesting candidate genes for cichlid speciation were mapped using SNP markers.

THE adaptive radiations of cichlid fishes of East Africa are well-known examples for rapid diversification and explosive speciation. More than 2000 species are phylogenetically very closely related since they originated within extremely short evolutionary time spans (Meyer *et al.* 1990; Meyer 1993; Kornfield and Smith 2000; Verheyen *et al.* 2003; Kocher 2004; Salzburger and Meyer 2004; Salzburger *et al.* 2005). Astonishingly, large numbers of species make up the three species flocks, each composed of hundreds of species, in lakes Victoria, Malawi, and Tanganyika (Fryer and Iles 1972). Despite the huge phenotypic diversity displayed by each of the species flocks, molecular phylogenetic studies on this problem revealed that many of the species evolved similar morphologies convergently in each of these three adaptive radiations (Meyer *et al.* 1990; Kocher *et al.* 1993; Meyer 1993; Stiassny and Meyer 1999). These striking phenotypic similarities among cichlid fishes from different species flocks that have evolved in parallel make the study of the underlying genetic architecture of cichlids particularly interesting.

The observed redundant patterns in the evolutionary diversification of cichlid fishes support the view that the three large East African lakes are a natural experiment of evolution, in which this parallel evolution might ultimately help to better understand the processes that led to the repeatedly evolved patterns of diversification. Particularly in the species of cichlids in lakes Victoria and Malawi, adaptive radiations are very young and genetically extremely similar. Comparative studies on the genomic organization of these closely related yet morphologically diverse fishes will help to unravel the genetics of speciation (Kocher 2004; Albertson and Kocher 2006; Hoegg *et al.* 2007). Investigation of the molecular basis of those different phenotypes, *i.e.*, the genetic and transcriptional changes that underlie differences among organisms, can be achieved through detailed comparisons of genome and transcriptome scans also including candidate gene approaches (Streelman and Kocher 2000; Braasch *et al.* 2006; Salzburger *et al.* 2007, 2008; Gerrard and Meyer 2007). For example, the gene for *long wavelength-sensitive opsin* (*Lws*) has been reported to be involved in sympatric speciation through ecological adaptation and mate choice of cichlids (Carleton *et al.* 2005; Maan *et al.* 2006; Terai *et al.* 2006; Seehausen *et al.* 2008), while the *microfibril-associated glycoprotein* (*Mfap4*) is a good candidate for examining species differences with regard to jaw development (Kobayashi *et al.* 2006).

Species-specific linkage maps have recently become established as important genetic tools in an effort to gain more detailed knowledge of genotype–phenotype

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relationships (Albertson *et al.* 2003; Erickson *et al.* 2004; Albertson and Kocher 2006). The latter approach is known as quantitative trait locus (QTL) scan, which makes use of the linkage disequilibrium created through experimental crosses between different species or laboratory strains (Falconer and Mackay 1996; Lynch and Walsh 1998). The ability to produce fertile interspecific crosses among some of these species of cichlids (Crapon de Caprona and Fritzsch 1984) and the general popularity of captive breeding support the establishment of cichlid fishes as a model system in comparative evolutionary genomic research.

The cichlid species *Astatotilapia burtoni* that occurs in Lake Tanganyika and in the surrounding river systems exhibits a rather generalist life style and is likely to represent a relatively ancestral type of cichlid (Meyer *et al.* 1991; Salzburger *et al.* 2005). Its phylogenetic placement between the species flock of ±500 endemic species in Lake Victoria and ≤1000 endemic species in Lake Malawi makes it an interesting species to study in this regard (Meyer *et al.* 1991). Since *A. burtoni* occupies a crucial phylogenetic position at the base of the extremely species-rich tribe of cichlids, the Haplochromini (Salzburger *et al.* 2002), which make up the large radiations of lakes Victoria and Malawi, its genome can serve as a sort of baseline from which comparisons to the endemic cichlids of these lakes will be particularly insightful. Given these close genetic affinities, most of the genomic resources developed for *A. burtoni* will also be applicable to the large haplochromine cichlid species flocks from lakes Victoria and Malawi.

For *A. burtoni*, a BAC library (Lang *et al.* 2006) as well as expressed sequence tags (ESTs) have been generated (Salzburger *et al.* 2008), and cDNA microarrays are available as well (Renn *et al.* 2004; W. Salzburger, H. A. Hofmann and A. Meyer, unpublished results). In addition, there is detailed knowledge on *Hox* genes (Hoegg and Meyer 2005; Hoegg *et al.* 2007), *Para-Hox* genes (Siegel *et al.* 2007), and several other genes related to coloration (Braasch *et al.* 2006; Salzburger *et al.* 2007) and fertilization (Gerrard and Meyer 2007) for this key species. Genomic resources available from other cichlids include the tilapia *Oreochromis niloticus* (BAC library: Katagiri *et al.* 2001; genetic maps: Kocher *et al.* 1998; Katagiri *et al.* 2005; Lee *et al.* 2005), *Haplochromis chilotes* (BAC library: Watanabe *et al.* 2003; ESTs: Watanabe *et al.* 2004; cDNA microarrays: Kobayashi *et al.* 2006), and *Metriaclima zebra* (Di Palma *et al.* 2007; ESTs: Salzburger *et al.* 2008; genetic map: Albertson *et al.* 2003). Recently, the National Institutes of Health has committed to sequencing four cichlid genomes. A detailed description of genomic resources developed for cichlid fishes can be found at http://www.cichlidgenome.org/.

AFLPs and microsatellite loci (also termed SSR) are the most common markers used in the development of linkage maps and QTL studies. Microsatellites are preferable because of their codominant nature and extremely high degrees of intraspecific allele polymorphism, which makes them most effective. On the other hand, their generation requires high costs and is time consuming (reviewed in Erickson *et al.* 2004). Most linkage maps based on microsatellites have been constructed for economically important fish species, such as the Atlantic salmon (Gilbey *et al.* 2004), rainbow trout (Sakamoto *et al.* 2000), European sea bass (Chistiakov *et al.* 2005), and Nile tilapia (Kocher *et al.* 1998; Lee *et al.* 2005), to search for loci that affect commercially important traits. Research in the fields of ecology and evolution has recently begun to focus on identifying the genetic basis of adaptive trait evolution especially in natural populations of nonmodel organisms. The past decade has seen a proliferation of studies that employ linkage maps together with QTL approaches to shed light on evolutionary processes, for example, the parallel evolution of benthic and limnetic forms in threespine sticklebacks (Peichel *et al.* 2001; Colosimo *et al.* 2004; Miller *et al.* 2007) and reduction of eyes and pigmentation in the Mexican cavefish, *Astyanax mexicanus* (Protas *et al.* 2007).

Here we report on the construction of a microsatellite linkage map of the cichlid fish *A. burtoni* based on an F_2 intercross derived from two inbred laboratory strains. We identified 25 linkage groups (LGs). The map also incorporates some EST-based markers and nuclear genes from sequenced BAC clones, *e.g.*, the seven reported *Hox* genes and the two paralogs of *Pdgfr*β, a gene involved in coloration (Braasch *et al.* 2006). This linkage map will thus provide a useful future tool for studying the genetic basis of adaptive traits that played a major role in the rapid diversification of cichlid fishes.

MATERIALS AND METHODS

Experimental crosses: We crossed an *A. burtoni* female derived from our University of Konstanz stock with a male stemming from a laboratory stock that originated in the laboratory of Russell D. Fernald at Stanford University. It is now also held at the laboratory of Hans A. Hofmann (University of Texas at Austin). The stocks are originally from the Tanzania and Zambia regions of Lake Tanganyika, respectively. The resulting F_1 generation was raised to sexual maturity, and groups of several females with one or two males were established for the F_1 intercross. Young fry of the F_2 generation, usually consisting of 10–50 individuals, were taken from the mouths of F_1 females. Genotyping at 10 microsatellite markers revealed family relationships within each group. The final mapping population included 167 F_2 offspring through intercrossing one male with five different females, thus constituting a half-sib family. However, we first established linkage groups by genotyping a subset of the first 90 F_2 individuals that were born and then by adding the remaining 77 individuals for those markers with low LOD scores and/or those with some missing data. Loci were genotyped for an average of 106 individuals.

Microsatellite markers: A microsatellite-enriched library was prepared from *A. burtoni* DNA using a magnetic bead enrichment protocol and $(CA)_{15}$ and $(CT)_{15}$ probes (for a

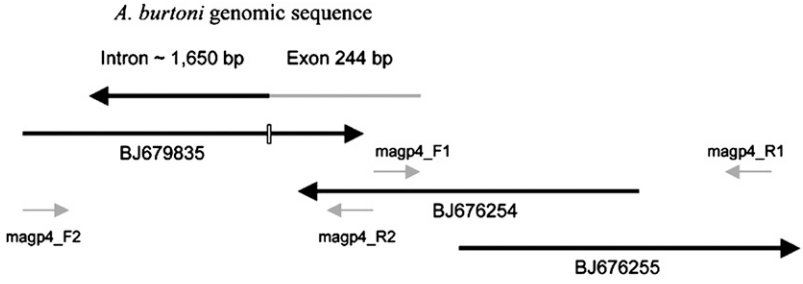

Figure 1.—Schematic of the amplification of the *Mfap4* gene from *A. burtoni*. Position of primers, alignment of EST clones of *H. chilotes*, and the location of an intron in *A. burtoni* derived from genomic DNA are shown. The clones and primers are not drawn to scale.

detailed description, see Sanetra and Meyer 2005). A total of 1156 clones were sequenced, and 683 clones (enrichment rate ~60%) contained repeat motifs (including ~10% duplicate clones). Primer sets were designed for 278 putative loci using the Primer3 software (Rozen and Skaletsky 2000). Markers were considered informative when at least one F_1 parent was heterozygous, which was the case for 147 of these loci.

Additional 191 microsatellite primer sequences were collected from available genomic resources for other cichlids and their usefulness for mapping the *A. burtoni* genome was tested. Finally, we were able to employ a total of 60 informative markers derived from the tilapia *O. niloticus* (Kocher *et al.* 1998; UNH106, UNH152, UNH130, UNH192), *Copadichromis cyclicos* (Kellogg *et al.* 1995; UNH002), *Astatoreochromis allaudi* (Wu *et al.* 1999; OSU9D, OSU13D, OSU19T, OSU20D), *Tropheus moorii* (Zardoya *et al.* 1996; TmoM5, TmoM7, TmoM27), *Pundamilia pundamilia* (Taylor *et al.* 2002; Ppun1, Ppun5-7, Ppun12, Ppun16, Ppun18-20, Ppun24, Ppun34-35, Ppun41), and *M. zebra* (Albertson *et al.* 2003; UNH2004, 2005, 2008, 2032, 2037, 2044, 2046, 2056, 2058, 2059, 2069, 2071, 2075, 2080, 2084, 2094, 2100, 2104, 2112, 2116, 2117, 2125, 2134, 2139, 2141, 2149, 2150, 2153, 2163, 2166, 2169, 2181, 2185, 2191, 2204). A comprehensive list of the markers used is provided in the supporting information, Table S1.

We also searched for microsatellite repeat motifs in 9375 nonredundant cDNA clones derived from a library using *A. burtoni* brains and mixed tissue including both sexes and all stages of development (Salzburger *et al.* 2008). For 21 microsatellite-containing cDNA clones, we developed PCR primers, 13 of which were polymorphic and gave reproducible results. GenBank accession numbers for these markers are as follows: Abur221, CN470695; Abur223, CN469772; Abur228, CN470764; Abur224, DY626128; Abur225, DY630453; Abur226, DY630491; Abur227, DY626763; Abur230, DY626468; Abur233, DY629660; Abur234, DY630828; Abur235, DY627273; Abur239, DY629088; and Abur240, DY630681. We compared these sequences to the cDNA and peptide database of medaka (*Oryzias latipes*), as the most closely related model organism to cichlids, using Blastview at http://www.ensembl.org/Multi/blastview.

Genotyping procedures: Microsatellites were amplified in 10-μl PCR reactions containing 10 mm Tris–HCl, 50 mm KCl, 1.5 mm $MgCl_2$, 0.2 mm dNTPs, 4 pmol of each locus specific primer, 0.8 units *Taq* polymerase (Genaxxon), and 10–30 ng genomic DNA. Forward primers were labeled with the following fluorescent dyes: 6-carboxylfluoresceine (6-FAM), hexachloro-6-carboxyfluorescein (HEX) and 2,7′,8′-benzo-5′-fluoro-2′,4,7-trichloro-5-carboxyfluorescein (NED). In addition, for 212 of the initially designed primer pairs, the M13 method for fluorescent labeling of PCR products (Schülke 2000) was used for economic reasons (see Table S1). With the latter method, 1 pmol of forward primer, 4 pmol of reverse primer, and 4 pmol of M13 6-FAM- or HEX-labeled primer were used. For the second round of genotyping, we used a PCR multiplexing kit (Qiagen) to amplify three to five loci in a single PCR reaction in a 12-μl volume containing 6.25 μl of 2× Qiagen Multiplex PCR Master Mix and 1.25 μl of a mix of primers. Final concentration of primers was 0.2 μm. All PCRs were run on a Perkin Elmer (Norwalk, CT) GeneAmp PCR 9700.

Three basic temperature protocols, depending on labeling method and multiplex scheme, were used: for forward primer labeling, one cycle of 3 min at 94°, 35 cycles at 94° for 30 sec, 48°–58° for 30 sec, 72° for 90 sec, and a final extension step at 72° for 10 min; for *M13 primer labeling*, one cycle of 3 min at 96°, 5 cycles at 96° for 30 sec, 62°–56° for 30 sec, 72° for 30 sec, 35 cycles at 96° for 30 sec, 58°–53° for 30 sec, 72° for 30 sec, and a final extension step at 72° for 10 min; and for multiplex PCR, 95° for 15 min, 35 cycles at 94° for 30 sec, 50°–60° for 90 sec, 72° for 60 sec, and a final extension period of 30 min at 72° (for details see Table S1). PCR products for four to six loci were combined with a mixture of ABI Genescan-500 ROX size standard and were analyzed with an ABI 3100 Automated Sequencer (Applied Biosystems). More details on fluorescent dye labeling, multiplexing schemes, and annealing temperatures (T_a) for each locus are given in Table S1. Allele sizes were scored with the Genotyper 3.7 (Applied Biosystems) software package and transferred to an electronic spreadsheet.

Type I markers: Several clones containing interesting candidate genes have been sequenced from the BAC library of *A. burtoni* (Lang *et al.* 2006). These clones incorporate the homologous sequences of *Pdgfr*β (Braasch *et al.* 2006) and all *Hox* genes reported in *A. burtoni* (Hoegg *et al.* 2007). Putative microsatellites were derived from the clone sequences with the Tandem Repeat Finder v. 3.2.1 software, so that two informative markers were obtained from BAC clone 26M7 containing *Pdgfr*βa (Abur209, -212) and one for 20D21 containing *Pdgfr*βb (Abur218). The corresponding BAC clones (clone number, accession number) for *HoxAa* (116M8, EF594313), *HoxAb* (150O18, EF594311), *HoxBa* (170E12, EF594310), *HoxBb* (34I18, EF594314), *HoxCa* (103K21, EF594312), *HoxDa* (32B18, EF594315), and *HoxDb* (19E16, EF594316) each yielded one to three polymorphic microsatellite markers. A tetranucleotide ATCT repeat was used for marker development of clone 99M12 (Siegel *et al.* 2007), which includes *ParaHoxC1*, representing a dispersed *Hox*-like gene cluster.

A homolog of the human *microfibril-associated glycoprotein 4* (*Mfap4*) has been reported from cichlid EST clones derived from *H. chilotes* (Kobayashi *et al.* 2006). We used three of these clones (accession nos. BJ679835, BJ676254, and BJ680594) to form 1214 bp of continuous sequence (Figure 1). We initially designed two pairs of primers to amplify from genomic DNA of *A. burtoni*: Magp4_1F (5′-TCAGACCTCCACCAAACAGTC) and Magp4_1R (5′-TCCCTGAAGACCATCAGCAT), spanning 501 bp of clones BJ676254 and BJ680594, and Magp4_2F (5′-CGGTGCAGGTG TACTGTGAC) and Magp_2R (5′-ACTGCACAGGACGGATC TTC) covering 544 bp of clone BJ679835. After identifying a SNP in an intronic region, two additional primers were designed from the *A. burtoni* sequence for genotyping: SNP_5F

(5′-GGCTTGTCTCATGTGCCTTC) and SNP_6R (5′-AC CAGCTGTCCTGGTCTTTTG), yielding a 339-bp fragment.

Lws has been characterized from a BAC library of *H. chilotes*. Numerous primers to amplify upstream and downstream regions of the gene are available (Terai *et al.* 2006). We used the following primers to amplify this region from genomic DNA in *A. burtoni*, all resulting in ~1-kb fragments: LWSB_LF and LWSB_R1, LWSB_F2 and LWSB_R3, LWSB_F3 and LWSB_R4, LWSB_F4 and LWSB_R5, LWSB_F8 and LWSB_R9, and LWSB_F9 and LWSB_R10.

The primers AroI_Ex7F (5′-GGTGATCGCAGCTCCGGA CACTCTCTCC), AroI_Ex8R (5′-CCTGTGTTCAGAATGAT GTTTGTGC), and AroI_1600R (5′-GTACAGCTAAAGGTT CGGGTC), for amplification of 600–1200 bp of *ovarian cytochrome P450 aromatase* (*Cyp19a1*), were designed using a genomic sequence from tilapia deposited in GenBank (AF472620) and using sequences from Lake Malawi cichlids at http://www.ncbi.nlm.nih.gov/blast/mmtrace.shtml (D. T. Gerrard and A. Meyer, unpublished results). Following SNP identification, the nested primers AroI_Fw (5′-ATGGCTG CATTCCACCAC) and AroI_Rv (5′-TTCTTCATGCTTCTGC TCCTC) were designed for *A. burtoni*, producing 447 bp of intron sequence located between exon 7 and exon 8.

The primary sequences of the three gene regions, *Mfap4*, *Lws*, and *Cyp19a1*, were amplified using the annealing temperatures of 55°, 58°, and 54°, respectively, and the following PCR conditions: one cycle of 3 min at 94°, 35 cycles at 94° for 30 sec, 55°–58° for 30 sec, 72° for 90 sec, and a final extension step at 72° for 10 min. Resulting sequences of *A. burtoni* were screened for SNPs in the parents of the mapping cross, and identified SNPs were then sequenced and scored using nested primers (with annealing of 55°) in 167 F_2 individuals.

Linkage mapping: Linkage groups, distances, and maker orderings were determined with LocusMap 1.1 (Garbe and Da 2003), JoinMap 4 (Van Ooijen 2006), and Map Manager QTX (Manly *et al.* 2001). Non-inheritance errors provided by Locusmap were checked by reevaluating the original chromatograms and either corrected or omitted from the data set. Most of these errors were due to rounding errors of a 1-base difference in allele size; others were classified as possible allele drop-outs or allele mutations. The assignment of markers to linkage groups was carried out by using a grouping LOD threshold of ≥4.0. This value was increased from the commonly used LOD score of 3.0 to minimize the risk of false linkage due to the large number of two-way tests being performed (Ott 1991). Maps of each of the linkage groups were obtained using a pairwise LOD threshold of 3 and a maximum recombination threshold of 0.4. The Kosambi mapping function, which accounts for double recombinations, was used to convert recombination frequencies to centimorgans for all analyses. The order of the markers was manually optimized to decrease the number of double recombinations. Markers that present shared alleles in the grandparents have reduced statistical power, since paternal/maternal homozygotes and heterozygotes cannot be discriminated in some F_2 families (*e.g.*, when one F_1 parent is homozygous for the shared allele). These markers could assume equally probable positions, and therefore their positions are represented as ranges in the map. The final map distances were calculated using Map Manager QTX. Graphics of the linkage groups were produced with the MapChart 2.1. software (Vorrips 2002).

RESULTS

Polymorphic microsatellites: We have characterized 278 new microsatellite sequences from genomic DNA of *A. burtoni*, 225 of which could be used to amplify PCR products of the expected size. One hundred forty-seven markers were informative (at least one grandparent heterozygous), while 49 were not variable in this mapping cross. The remaining 28 loci showed banding patterns that were difficult to interpret and thus could not be reliably scored. An additional 60 microsatellite markers were derived from prior studies that employed microsatellite markers in cichlids (Kellogg *et al.* 1995; Zardoya *et al.* 1996; Kocher *et al.* 1998; Taylor *et al.* 2002; Albertson *et al.* 2003). Most of the microsatellites used consist of pure and compound dinucleotide tandem repeats, composing mainly CA and. to a lesser extent, CT repeat motifs. In addition, there were 13 tetranucleotide loci developed from the genus Pundamilia (Taylor *et al.* 2002) and one from a BAC clone containing *ParaHoxC1*. An investigation of the relationship between repeat length and rate of polymorphism showed no significant correlation; however, no repeats shorter than eight times were used initially.

***A. burtoni* linkage map:** Significant linkages were identified for 204 genetic markers, including 191 microsatellite loci and 13 type I (gene) markers (Figure 2). Only six markers (Abur58, Abur147, Abur190, UNH002, UNH2075, and UNH2185) could not be linked to any other marker (97% of markers could be linked). We found 25 linkage groups with the number of markers per group ranging from 3 (LG19, -24, -25) to 18 (LG1). The largest linkage groups were LG1 and LG2 with 109.7 and 91.5 cM, respectively. The *A. burtoni* karyotype ($2n = 40$) shows at least two large subtelocentric-telocentric chromosomes, although karyotypic size variation among chromosomes was not as pronounced when compared to Tilapia and Sarotherodon (Thompson 1981).

The total sex-averaged length of the map was 1249.3 cM. Marker spacing was on average 6.12 cM, with the largest distance being 33 cM. Markers that had a common allele in both grandparents had reduced mapping power (since the origin of the allele cannot be identified) and presented a range of equally likely positions, which are indicated in Figure 2. Since the genome size of this species has been reported to be 0.97 pg (Lang *et al.* 2006), which equals ~950 Mb, we estimated the physical-to-map distance as 760 kb/cM.

***Hox* gene clusters:** In *A. burtoni*, seven *Hox* gene clusters have been detected by means of BAC library screening and sequencing of positive clones (Hoegg *et al.* 2007). The mapping results using microsatellite flanking regions revealed a distinctive distribution of *Hox* clusters throughout the genome. *HoxAa*, *HoxAb*, *HoxBa*, and *HoxBb* were mapped to LG15, LG4, LG16, and LG20, respectively. While *HoxCa* was assigned to LG11, *HoxDa* and *HoxDb* mapped to LG22 and LG14, respectively. Overall, this pattern supports the hypothesis of the fish-specific genome duplication (FSGD) because all *Hox* clusters are found on different chromosomes (Hoegg and Meyer 2005; Meyer and van de Peer 2005). *ParaHoxC1* is on LG18 with no association

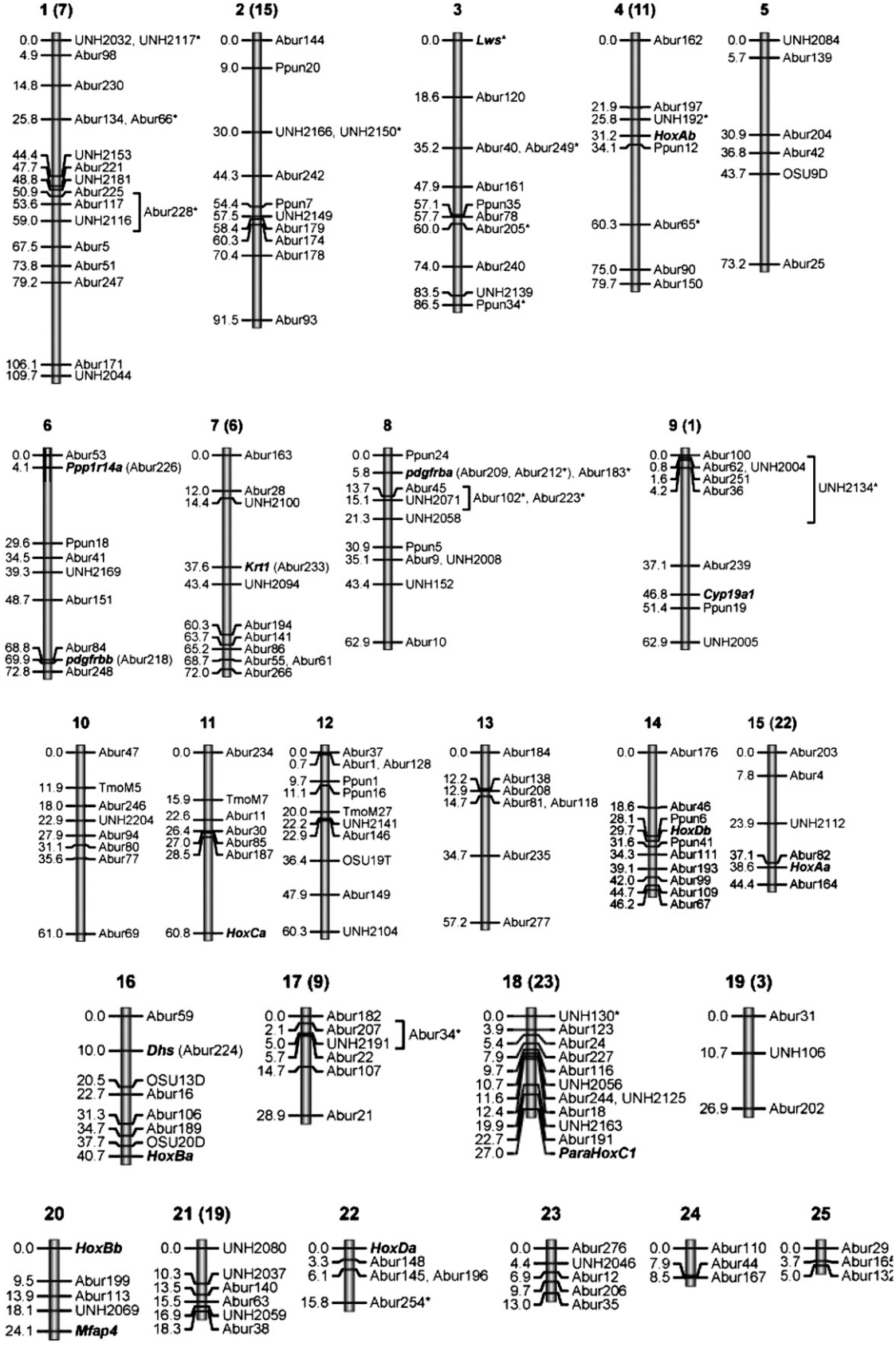

FIGURE 2.—Linkage map of *A. burtoni* comprising 25 linkage groups. Corresponding linkage groups for tilapia are in parentheses as inferred from shared markers among the three African cichlid maps. Distances in Kosambi centimorgans are indicated at the left of each linkage group. Asterisks indicate loci at which the crossed F_0 grandparents share a common allele and paternity/maternity of the particular allele could not be identified unambiguously for all six half-sib F_2 families.

with one of the other conventional *Hox* clusters, corroborating the idea that these *Hox*-like genes constitute dispersed homeobox genes forming novel clusters somewhere else in the genome (Siegel *et al.* 2007).

***Platelet-derived growth factor receptor* β (*Pdgfr*β):** Two paralogons of this gene are known to be present in cichlids due to the FSGD (Braasch *et al.* 2006). The mapping of adjacent microsatellite markers of type $(GT/CA)_{12–15}$ from corresponding BAC clones showed the location of *Pdgfr*β*a* on LG8, while *Pdgfr*β*b* was mapped to LG6. In the same BAC clone, the two markers Abur209 and Abur212 flank the *Pdgfr*β*a–Csf1r* tandem, spanning ~50 kb between them. It is thus not surprising that they are regarded as identical in the map. The observation of *Pdgfr*α (see the above treatment on *ParaHoxC1*) and the A- and B-copies of *Pdgfr*β occurring on different linkage groups, as in *Hox*-genes, favors the origin of this gene family by whole-genome duplication and not by tandem duplication.

***Long wavelength-sensitive opsin* (*Lws*):** In the sequence upstream of the *Lws* gene using the primers LWSB_F2 and LWSB_R3, we found a SNP at position 231 (using nested primers) in the F_0 grandparents of the mapping cross. The male parent was CT heterozygous and the female parent was TT homozygous, thereby permitting linkage analyses (yet with reduced statistical power) through genotyping of F_2 individuals. The analysis revealed that this gene, which is believed to be important for color vision and probably speciation by female mate choice (Terai *et al.* 2006), is located on LG3 in the linkage map of *A. burtoni*. *Lws* therefore does not appear to be linked to any other candidate gene investigated in this study.

***Microfibril-associated glycoprotein 4* (*Mfap4*):** Amplification from genomic DNA with the two primer pairs directly derived from EST clones of *H. chilotes* led to the discovery of an intronic region in sequence BJ679835 (Figure 1). Since the expected size of the product from cDNA was 544 bp and the observed size was 2.2 kb, the size of the intron could be estimated to be ~1.65 kb in length. Within this intron we identified a SNP at position 392 in the sequence given by primer Magp4_2R when aligned to clone BJ679835. At this site the male parent was TT homozygote and the female parent was CC homozygote, while all F_1 individuals were CT heterozygotes, as expected. We also compared the amplified coding regions between *H. chilotes* and *A. burtoni* and discovered a surprisingly large number of 15 substitutions in only 195 bp. For SNP genotyping of the F_2, we used nested primers in the intron region yielding a shorter fragment of 339 bp, subsequent linkage analyses of which showed the *Mfap4* locus to be positioned on the small LG20 (five markers, 24.1 cM). The latter also comprises the *HoxBb* cluster and the marker UNH2069, which shows an associated QTL for jaw morphology in the Malawi cichlid Metriaclima (Albertson *et al.* 2003).

***Ovarian cytochrome P450 aromatase* (*Cyp19a1*):** We were able to identify two SNPs (AF472620:g.4167A>C and AF472620:g.4440G>T) in the F_0 parental DNA of the *A. burtoni* mapping cross: one in the intron bridging exons 7 and 8 and the second one in exon 8. The male parent was homozygous for cytosine and guanine at positions g.4167 and g.4440, respectively, while the female parent was homozygous for adenine and thymine in these same positions. Linkage analysis with microsatellite markers using an F_2 intercross revealed the map location of *Cyp19a1* on LG9 in *A. burtoni*. In the Nile tilapia, *Cyp19a1* was found in the vicinity of the presumed *SEX* locus on LG1 (Lee and Kocher 2007). This linkage group corresponds to the original LG6 reported by Albertson *et al.* (2003) for Metriaclima, but this has now been renamed in accordance with the tilapia map as LG1 (Albertson *et al.* 2005).

EST-linked microsatellites: Thirteen informative microsatellite markers (Abur221, 223–228, 230, 233–235, 239–240; Table S1) were produced from EST clones of *A. burtoni*. These markers were widely distributed on several different linkage groups, such as LG1, -3, 6–9, -11, -13, -16, and -18, making them especially suitable for genome scans to discover functional polymorphisms. Comparisons of *A. burtoni* EST clones with known sequences of medaka revealed a few homologies with protein-coding genes. The sequence adjoining marker Abur224 on LG16 was indicated as part of the transcript of *deoxyhypusine synthase* (*Dhs*), which occurs in a single copy in medaka and is essential for cell viability. Despite the short overlap of 42 bp and therefore the high *e*-value [$2.2e^{-11}$; percentage identity (PID): 79%), alignment structure and orientation to the poly(A) tail with the microsatellite in the 3′-UTR strongly support this assumption. Abur226 on LG6 ($6.4e^{-42}$; PID 78%) corresponds to *protein phosphatase 1* regulatory subunit 14A (*Ppp1r14a*), a cytosolic inhibitory protein of PP1 with a molecular weight of 17 kDa. Abur233 on LG7 ($5.6e^{-50}$; PID 77%) was found adjacent to a member of the protein family *keratin, type I* (cytoskeletal cytokeratin), accounting for the keratin filaments in epithelia. Teleost fish show an excess of keratin type I over type II genes; thus as many as 17 type I gene members are present in medaka. For more annotations of EST sequences from this library, see Salzburger *et al.* (2008).

Comparison of the *A. burtoni* and the *M. zebra/Labeotropheus fueleborni* maps: A genetic linkage map is available from a hybrid cross of two closely related Lake Malawi cichlids, *L. fueleborni* and *M. zebra* (Albertson *et al.* 2003). It contains 127 microsatellite markers, 33 of which we were able to use as informative markers in the linkage map of *A. burtoni*. A comparison of the two maps revealed good concordance in some parts, in that all markers located in a single linkage group in Lake Malawi cichlids, *e.g.*, LG2 with UNH2037, -2059, and -2080, were found on LG21 of *A. burtoni* as well. In addition, numerous markers appeared jointly—*i.e.*, in synteny—in the same order (with the exception of

TABLE 1

Oxford plot comparing the linkage maps of *A. burtoni* and *M. zebra/L. fueleborni*

	M. zebra/L. fueleborni																							
A. burtoni	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	6																							
2																3								
3												1												
4																								
5			1																					
6											1													
7					2																			
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UNH2116 and UNH2181) on differently named linkage groups in the two maps, suggesting conservation of genomic regions in those linkage groups across different species of cichlids. It appears that *A. burtoni* linkage groups LG1, -2, -7, -8, -9, -12, -18, and -21 are homologous to LG1, -16, -5, -4, -6, -3, -10, and -2, respectively, in the Lake Malawi cichlid map (Albertson *et al.* 2003) when using the criterion of at least two shared markers per LG. The observed correspondences of microsatellite markers are reported in the Oxford plot in Table 1.

Comparison between the *A. burtoni* and the tilapia (*Oreochromis* spp.) map: We initially screened 51 microsatellite markers from the available linkage maps of tilapia (Kocher *et al.* 1998; Lee *et al.* 2005) for their use in linkage mapping of *A. burtoni* and found rather low levels of polymorphism in our testcross, although amplification success was ~50%. Thus, only a small number of markers are shared between the two maps, namely UNH106, UNH2191, UNH192, UNH2150, UNH2166, and UNH130, that could be used for comparison. In the tilapia map containing 24 linkage groups, markers on LG3, -9, -11, -15, and -23 indicated correspondence of these linkage groups to LG19, -17, -4, -2, and -18, respectively, in the *A. burtoni* map. Due to the small number of tilapia microsatellites that were successfully mapped, the correspondence of LGs was determined with the sharing of a single marker, with the exception of LG2, which shares two markers with LG15 of tilapia.

SNP mapping of *Cyp19a1* in *A. burtoni* mapped this gene to LG9, which therefore might be homologous to LG1 in tilapia, which contains the genes *SEX* and *Cyp19a1* (Lee and Kocher 2007). In accordance with that, Albertson *et al.* (2005) have renamed LG6 from an earlier article on Metriaclima (Albertson *et al.* 2003) to LG1 as in tilapia. This might suggest that similarities among all three East African cichlid genetic maps could be used to find probable synonymies of linkage groups. We attempt to reconcile the nomenclature of the present genetic linkage maps of cichlids (supporting information, Table S2). Also, using the tilapia map as a standard, some of these relationships were included in Figure 2.

DISCUSSION

Here we present the third genetic map of a cichlid fish. *A. burtoni* occurs in Lake Tanganyika and its surrounding rivers. It significantly adds to the knowledge of previous linkage maps for the generalist and geographically widespread tilapia, *Oreochromis* spp. (Kocher *et al.* 1998; Lee *et al.* 2005), and the specialist Lake Malawi endemic *M. zebra/L. fueleborni* (Albertson *et al.* 2003). In the *A. burtoni* map, we identified linkages

among 204 genetic markers, mainly microsatellites and 13 type I (gene) markers, which were assigned to 25 linkage groups. The difference in size of the linkage groups corresponds quite well with chromosome morphology reported from investigations of the karyotype. While most chromosomes are relatively small and of metacentric-submetacentric or metacentric type, there are also four large subtelocentric-telocentric chromosomes in the karyotype (Thompson 1981). The *A. burtoni* karyotype consists of 20 chromosomes; it is therefore expected that some of the linkage groups will coalesce with the addition of more markers. Lake Malawi cichlids, on the other hand, have a slightly higher chromosome number ($N = 23$) (Thompson 1981) in very good agreement with the 24 linkage groups found by Albertson *et al.* (2003) for an interspecific cross between *M. zebra* and *L. fueleborni*. The current map of tilapia, although one of the most detailed fish linkage maps, still contains two linkage groups more than is expected from the 22 chromosomes of the karyotype (Lee *et al.* 2005).

There is a quite good correspondence between the *A. burtoni* and the Lake Malawi genetic maps (Table 1). Synteny between microsatellite markers was common while only a few unexpected syntenies among shared markers were found, such as UNH2084 that was mapped to LG5 instead of LG12 in *A. burtoni*. Those marker locations might indicate genomic rearrangements such as translocations of chromosomal sections. The synteny of markers used for the map of tilapia could not be directly explored in *A. burtoni* possibly because of the considerable evolutionary distance (~15 MY) between these species. This is the most probable explanation for the observation that a large portion of the PCR reactions for particular markers did not work. Many of the markers that did amplify did not show polymorphism in the *A. burtoni* mapping cross. Hence, the hope of a wide applicability for the tilapia map for the >2000 species of cichlid fishes in the East African lakes (Lee *et al.* 2005) might have been somewhat optimistic. Nevertheless, by using the combined information from the three maps of East African cichlids, many linkage groups could be interpreted as being orthologous with some linkage groups of the tilapia map on the basis of shared markers (see Table S2 and Figure 2).

Comparison of the map location of the gene for *ovarian cytochrome P450 aromatase* (*Cyp19a1*) on LG1 in tilapia (Lee and Kocher 2007), together with the overlapping markers used in *M. zebra* and *A. burtoni* (Table S2), suggests that the orthology of LG6 in *M. zebra* and LG9 in *A. burtoni* to that tilapia LG1 is highly likely (see also Albertson *et al.* 2005). *Cyp19a1* is involved in sex differentiation of mammals and could also be important in determining sex in vertebrate species that lack sex chromosomes. However, in the Nile tilapia, this gene was found 27 cM away from the presumed sex-determining locus on LG1, calling into question its function as a master control gene for sex determination. In general, the sex locus in tilapia (*O. niloticus*) behaves like an XY male heterogametic system (Lee *et al.* 2003; Lee and Kocher 2007). On the other hand, Lee *et al.* (2004) found microsatellite markers consistent with a WZ (female heterogametic) system on LG3 in *O. aureus*. Thus, the mechanism of sex determination appears highly variable among species of African cichlids—as appears to be the case in fishes more generally (Volff *et al.* 2007). The occurrence of *Cyp19a1* on LG9 in *A. burtoni* raises the possibility that a sex-determining factor is located on this chromosome. It is known, however, that alternative chromosomal sex-determining mechanisms have evolved independently in closely related fish species (Takehana *et al.* 2007; Henning *et al.* 2008).

Using microsatellites from BAC clone sequences, we were able to map all seven clusters of *Hox* genes that have been reported from *A. burtoni* (Hoegg *et al.* 2007) as well as the *ParaHoxC1* cluster (Siegel *et al.* 2007). The surprising variation in *Hox* cluster numbers among vertebrates has been widely used to study the evolution of vertebrate genomic organization (Hoegg and Meyer 2005). While the phylogenetic timing of the first two rounds of genome duplications (that also resulted in the *Hox* cluster duplications) during the chordate-tetrapod evolution is still somewhat debated, the evidence is solid that the eight Hox clusters in ray-finned fish originated through a whole-genome duplication (the FSGD or 3R hypothesis) (Malaga-Trillo and Meyer 2001; Hoegg *et al.* 2004; Meyer and van de Peer 2005). In accordance with the FSGD hypothesis, all *Hox* clusters were found to be scattered throughout the genome of *A. burtoni*. Similarly, zebrafish have seven *Hox* clusters on seven different chromosomes (Postlethwait *et al.* 1998), but their *Db* cluster was lost instead of the *Cb* cluster in *A. burtoni* (Hoegg *et al.* 2007). The *ParaHoxC1* paralogon mapped to LG18, which does not carry any other genes of the *Hox* complex. Sequence comparisons showed that *ParaHoxC1* and its 3′ adjoining genes of *Danio rerio* are located on chromosome 20 (Siegel *et al.* 2007). In general, synteny of the duplicated genes in teleosts seems to be less conserved in the *ParaHox* genes compared to the *Hox* genes.

One important cause for the vast amount of cichlid diversification might be related to the abundance of color morphs in different populations that through mate choice and male–male competition might lead to speciation (*e.g.*, Turner and Burrows 1995; Seehausen and Schluter 2004). Therefore, the mapping of candidate genes involved in fish coloration would seem to aid in the study speciation processes in cichlids. For example, the orange blotch color pattern in *M. zebra*, which is expressed mainly in females, has been mapped and candidate genes for this pigmentation phenotype have been identified (Streelman *et al.* 2003). We mapped two paralogons of the tandems *Pdgfr*β-*Csf1r* and

one of *Pdgfrα-Kita*, a family of receptor tyrosine kinase genes that have been shown to influence coloration in teleosts (Braasch *et al.* 2006; Salzburger *et al.* 2007). While the *kit* gene is essential for the development of neural-crest-derived dark melanocytes in mammals and zebrafish (Parichy *et al.* 1999), *Csf1r* promotes the development of yellow xanthophores in zebrafish (Parichy and Turner 2003), and there is evidence for its role in the development of cichlid egg spots (Salzburger *et al.* 2007). The location of the *Pdgfrβ* paralogons on two different linkage groups of *A. burtoni* (*Pdgfrβa* on LG8 and *Pdgfrβb* on LG6) lends further support to the hypothesis of the fish-specific genome duplication. In general, the teleost A-paralogon has retained a longer stretch of synteny with the single copy of the tetrapod locus compared to the B-paralogon (see Braasch *et al.* 2006). It has therefore been suggested that the B-paralogon underwent functional divergence (possibly neofunctionalization) of the cell-surface receptors. These duplicated receptor genes would be important targets for future QTL studies of cichlid coloration patterns.

Divergent evolution of the visual system is a likely mechanism to explain incipient speciation and diverse patterns in the male breeding coloration in cichlids (Terai *et al.* 2006). *A. burtoni* is a close relative of the sibling species pairs in the genus Pundamilia, for which the sensory drive hypothesis (differences in male coloration evolving as a consequence of divergent visual sensitivities) has been suggested to involve the gene for *long wavelength-sensitive opsin* (*Lws*) (Seehausen *et al.* 2008). This gene shows the highest variability among cichlid opsins and appears to be under strong divergent selection at least in the Lake Victoria species flock (Carleton *et al.* 2005). This gene mapped in *A. burtoni* to LG3, which corresponds to LG5 in tilapia that carries the genes for the *Blue Opsin* and for *c-ski I* (Lee *et al.* 2005). Remarkably, the latter marker has been found to be in close association with the orange blotch color polymorphism in *M. zebra* (Streelman *et al.* 2003). It will thus be interesting to examine whether other linked genes for coloration co-occur on that chromosome.

Apart from color, evolutionary diversification in cichlids is also believed to be driven by trophic specialization and associated altered jaw morphologies and tooth shape in different species (*e.g.*, Kocher 2004; Albertson and Kocher 2006; Streelman and Albertson 2006). A QTL study by Albertson *et al.* (2003) focused on these feeding adaptations and suggested that the oral jaws of Lake Malawi cichlids evolved in response to strong divergent selection. Closely related species of Lake Victoria cichlids such as *H. chilotes* and sp. "Rock Kribensis" can exhibit quite divergent types of jaws. A conspicuous difference in expression of the *Mfap4* gene (encoding a microfibril-associated glycoprotein) was recently discovered in these species (Kobayashi *et al.* 2006). *Mfap4* therefore may be partly responsible for the morphological differences among cichlid species. *Magp4* is also known to be involved in a human heritable disease, the Smith–Magenis–Syndrome, which results in a characteristic phenotype with a flattened mid-face and striking jaw and forehead (Zhao *et al.* 1995). The *ci-Mfap4* mapped to the small LG20 in *A. burtoni*. It also contains the *HoxBb* cluster and three anonymous microsatellite markers. The corresponding (shared marker UNH2069) LG20 in *M. zebra* also carries a QTL for differences in lower-jaw width (Albertson *et al.* 2003). Another candidate gene for craniofacial diversity, *bone morphogenetic protein 4* (*bmp4*), is located on LG2 (renamed LG19 in Albertson *et al.* 2005) in *M. zebra* (which corresponds to LG21 in *A. burtoni*) and explains >30% of the phenotypic variation in the opening and closing levers of the cichlid lower jaw (Albertson *et al.* 2005).

Thirteen EST-based microsatellite markers derived from brain and mixed tissue libraries of *A. burtoni* (Salzburger *et al.* 2008) mapped to its entire genome. Homology searches using the medaka database resulted in three reliable hits for *deoxyhypusine synthase* (*Dhs*), *protein phosphatase 1* (*Ppp1r14a*), and a member of the keratin, type I protein family. The remaining EST-linked *A. burtoni* markers came from anonymous clones, for which no homology with known sequences could be established. Nevertheless, they might prove to be useful gene-associated markers for detecting signatures of divergent selection (Vasemägi *et al.* 2005). In particular, the broad taxonomic application range of at least some microsatellite markers in East African cichlids provides a rare opportunity to use such markers with a known chromosomal location for comparative studies on polymorphism and to examine the footprints of selection.

Genetic linkage maps are valuable genomic resources that have been widely used for a number of different applications, but particularly interesting may be their use in QTL approaches for evolutionary questions (Erickson *et al.* 2004; Slate 2005). One of the most debated questions in evolutionary biology is whether major genes play a key role in species differences or whether a large number of small changes bring about phenotypic diversification during speciation (Orr 2001). QTL studies have made significant contributions to this issue (*e.g.*, Hawthorne and Via 2001; Peichel *et al.* 2001; Albertson *et al.* 2003). In threespine sticklebacks, for example, it was shown that a major QTL causes large shifts in phenotyp such as the morphological differences seen between the sympatric benthic and limnetic forms (Colosimo *et al.* 2004). These first studies support the hypothesis that large effects in a small number of genes, more often than small effects in large numbers of loci, tend to bring about large phenotypic changes and might even cause evolutionary novelties. However, whether these first results will turn out to be typical is still unclear.

Studies in East African cichlids found new relationships between genomic regions involved in feeding

adaptations and jaw types (Albertson *et al.* 2003, 2005), including the discovery of a region explaining ~40% of the phenotypic variance in cichlid tooth patterning (cusp number) (Streelman and Albertson 2006). It is thus promising that whole-genome sequencing has been initiated for several species of cichlid fishes, including draft sequencing (5×) of tilapia (*O. niloticus*) and 2× coverage each of three haplochromine species (*A. burtoni*, Pundamilia, and *M. zebra* will be among these haplochrome cichlids whose genomes will be sequenced (http://www.cichlidgenome.org/) so that the medium-density genetic map of *A. burtoni* can be used with QTL analyses to ultimately identify evolutionarily important genes (those that are presumably selectively adaptive). Comparisons of the sequenced cichlid genomes will then give insights into the similarity or differences in the underlying molecular changes that caused their phenotypic divergence among closely related species or caused convergent similarities among more distantly related cichlid species.

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A Microsatellite-Based Genetic Linkage Map of the Cichlid Fish, *Astatotilapia burtoni* (Teleostei): A Comparison of Genomic Architectures Among Rapidly Speciating Cichlids

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TABLE S1

Loci names, accession numbers, primers sequences, fluorescent labels, product size of cloned fragment, and annealing temperatures

MICROSATELLITE	LG	Dye	T_a	Size/Author	Forward primer	Reverse primer
Abur1	12	HEX	55°	281	AAAGGCCTGTCATCAACTGG	GGTCCCCACAGGGTAAATG
Abur4	15	FAM	55°	320	TTAAGGCCACCGTAGATTCC	CTGGTAGCCTGGAGGTCAGC
Abur5	1	HEX	55°	210	TCACATTGTTCGTTTTGCAC	ACGCTCAACAGACAGCAATC
Abur9	8	FAM-M13	60°/58°	208	GGCCACACGCTTAATAGTCC	TGACTTATATTTGCATCCGTGTG
Abur10	8	FAM-M13	60°/58°	123	TCGAGCTCAGCTTTCTCACTC	GCAGCACTGATTGATGCTAGTC
Abur11	11	FAM	55°	219	GCCATGGCACCTTTAATATG	ACCCTTACATGCCCAAAAAG
Abur12	23	HEX	55°	129	TTCTCGGCCACCAATTTTAC	GGTGGGACGGGATGAGAT
Abur16	16	HEX	55°	400	TGGTCCGTTTAAATGTGTGC	TGGGAGGTTTAATAATTAGTGATG
Abur18	18	FAM	55°	391	CACGTGACGTCCTGATGAAC	GCCTACCTGTGGGATACAGC
Abur21	17	FAM-M13	62°/58°	201	AATGTCATGTGGCTTCTCACC	CTGCCTAGTTCTCCCTGAGC
Abur22	17	FAM	55°	130	TGAGGCATTTTTGTCAATCC	GCGTGCACACGTTAGTGG
Abur24	18	FAM-M13	62°/58°	144	GTTGCATGTAGCTTGCCATC	ATCCATCCACCCATTCACTC
Abur25	5	FAM	55°	210	CGAACCTCAAACGTCCTCTC	CTGCTCTGATGGAGTCCAAG
Abur28	7	HEX	55°	315	CGAAGCGCTTTAGTGGTTTC	CATCGCTCAGCTTTCTCCTC
Abur29	25	FAM	55°	294	GGCAGTTTCTTTTTAACAACATGTAA	AAAGGGACGAACGTGACATC
Abur30	11	FAM	58°	200	GGTGAGCTGCATACCACAAG	GTCAGCTGTGAGCGTCTGG
Abur31	19	FAM	55°	392	ACTGTGGAGACGTGCAAGG	AAACCTGTACGTCGCTGGAC
Abur34	17	FAM-M13	62°/58°	250	AACTCTGAGCAGCAACAGCA	TCTCAGTGAATCAGCATTCCA
Abur35	23	HEX	55°	373	AGGGCGAGAGACCCTGAC	CTGTTCTCTGAGGTCCTGACG
Abur36	9	FAM	55°	395	TCTCTGAAGGTGAGCCACAG	ACGCTGCCCCTCTTCATC
Abur37	12	HEX	55°	389	TCGTTTACTTTTATTTATTTCCACCTG	TCTCGACTCAGAGTGCTAATTG
Abur38	21	HEX	58°	280	GGGAAAGAAGCCGTTTGG	TTAATGCAGGAGAACAGACAAGG
Abur40	3	HEX	50°	320	TATTGTCATCCCAGGTCACG	TTGATGTCAGATTTTTCTTTAATTTG
Abur41	6	FAM	55°	133	TAAGTTTCTGGGCCTTGCTG	TGAAGGAAAGAAGCCCAAGC
Abur42	5	FAM-M13	62°/58°	316	TCGAGCTTCCTGCACTATTTC	AATCTGCATGACTGGGAGATG
Abur44	24	FAM	55°	227	GACCATTTGCCCGCTAAG	GAAACTCGATTGGCTGCTTC
Abur45	8	HEX	55°	380	AGCAGTGGATGTGGCAGAG	TACAGGATGTGCCCCTCTC
Abur46	14	FAM	55°	400	CCCCTGCACTCGTTTACTTTAG	GAAAACAGAACGCAATCCAAG
Abur47	10	HEX	50°	124	TCCAGAAACACAGTTGTGACG	TTTTGTTCAACATTTATGCCTTTAC

Abur51	1	FAM	58°	178	GGATGGCATGGACTCAGAAG	TTTTCACTTTCCCCATCTCAC
Abur53	6	HEX	55°	400	CGACATGAAGACGGCTAAAG	GCTCTCTGTGCTGTGGTTTG
Abur55	7	HEX	55°	412	CTTCAACGAACCTGCGAGTC	GAGGAGCTGCTGGAGAACTG
Abur58	unassigned	FAM	55°	350	GACCCGACGTAGGACAAAAC	AGCACACCTGTGTGTTCCTG
Abur59	16	FAM	55°	500	ACCGGTACACTCTCCCACAG	CAGTTGCAATAGGCTGAACG
Abur61	7	HEX	50°	131	GATTGGCCGAACACAGAAAG	ACCTGGGTGTAAAGCAGCTC
Abur62	9	FAM-M13	62°/58°	377	TGTCATTCAGGCAGCATTTC	ATGAGCCCAGATGGTGATTG
Abur63	21	FAM-M13	62°/58°	313	ATATGGCTGCCAATCACAGG	GTGTGCAACCATTACGCAAC
Abur65	4	HEX	55°	235	TCTTCCAACATGCACAGAGC	GTTAAGCCGTTTGGACAAGG
Abur66	1	FAM	55°	450	TTCTCGCCTTCCGACAAG	TCCGTCCTTATGGTGTGAGC
Abur67	14	FAM-M13	62°/58°	275	AACCTGGCAGAGCTGAAGTG	AGGTACCCCACGTGATGATG
Abur69	10	HEX	55°	392	ATGCGCGGTTTTTGTAGTG	TCCCCTCATCTGATTCTTGC
Abur77	10	FAM-M13	62°/58°	186	TCTGTGACGTTTGCCTCTCC	TTGTCTTTCTGCCAATGCTG
Abur78	3	FAM	58°	234	GCTGCCTTGCATTAAAGTCC	TATTGCAGACACCGTTCAGG
Abur80	10	HEX	55°	214	GCAGCTTGTTGGGAAGAAAC	CTGACTGAGCTGTCCCTGTG
Abur81	13	HEX	55°	383	ACTCAGCGTCTCCCAGTGTC	TCGGTCACTCACAAATGCTC
Abur82	15	FAM	55°	340	ACAAAGAGCATGCACAAATG	CAGGAGACACAGTGGGAGATG
Abur84	6	FAM-M13	62°/58°	327	CACCTGCTGTCCTTGAGATG	AGGTGAGTGTGGGCTCCTC
Abur85	11	FAM-M13	62°/58°	368	TGTCAAAGCTGGATTGATGG	ACTGCCCACAGCAGGAAG
Abur86	7	FAM-M13	62°/58°	131	ACCCTGCCAGGCTAAGTAAG	AAGGGAATAAGCGTCGAAGG
Abur90	4	HEX	55°	146	AACCAAGCCTGAAAATCCTC	GCCAGCTTCAGATGTGGTACTC
Abur93	2	FAM	50°	260	ACTTCTCAGTGAATCAGCATTTC	CAGGACGGACAGAACTTTGG
Abur94	10	HEX	55°	216	CCACCTCAGGGTGCTTTATAC	TCAGGAGGTCCAACTATCATCAC
Abur98	1	HEX	55°	224	TCACGCACTTCTTTGTTTGG	AAGGGACGTTGCTCAATCAC
Abur99	14	FAM	55°	300	ATCGTCACCGCAATAACCTC	CCGAGTACTCTGGAGCAAGG
Abur100	9	HEX	55°	428	CATTCCAATCCCCTGTGC	CTGCTCCAACTCTCCTGTCC
Abur102	8	FAM	55°	326	CCCTAGCTGCTTACCCTAACC	CACCTGCACAGGAAACACAC
Abur106	16	FAM-M13	62°/58°	238	AGATGAAACTGTCCAGGCAAC	ATGCGCCTCAAATAGAAACG
Abur107	17	FAM	55°	136	CTGGACTTTTGAGTCCCAGTG	CATCACCACCCGATACACAG
Abur109	14	FAM	55°	197	AGACAGCGTTGAACCCAGAG	CGACTTTGGCTTCCTCAAAC
Abur110	24	FAM	55°	326	CGAAGGGAGATGATAGGGAAG	AGTAGTACTTGCACAGAAGGAGAAG
Abur111	14	HEX	55°	330	ATTTGGACGAGGAGGATGAG	AAGTGTTTCCAGCGTTGGTC
Abur113	20	FAM	55°	399	GCCTGAGCACCCAGAAATAC	GCATCATTTGGCTCTTTTCC
Abur116	18	FAM	55°	477	CCGTGTGTGTTTGAGTGAGC	CATGGTTAGGGCATCTGTGG

Abur117	1	HEX	55°	320	GTTCGGTTGAGGTGACAGTG	GAGGAAGTATCCGGTTTGTATTG
Abur118	13	HEX	55°	138	CAGTCACACTGGGGTGTCG	AATGTGTGCATGTCCCTGTG
Abur120	3	HEX	55°	146	CTGCACAGGCCTGAAGAAC	TACAGCAAGAACGGCAGGAC
Abur123	18	HEX-M13	62°/58°	178	CACACTACAACAGCCTAGAGGAG	AAAGCTTTGGTGCATTACCG
Abur128	12	HEX-M13	62°/58°	129	GGCGTTCAGTGATGGTTAGG	TCTTCATCGCCAACCTCTTC
Abur132	25	HEX-M13	62°/58°	274	GCTCCTTACAGCTGGAGTGC	TCTGTGCTCAGGTGTCGATG
Abur134	1	HEX-M13	62°/58°	311	AGAGACATGCACAGGCACAC	TGAGCCAGTGCTTAACCTTG
Abur138	13	HEX-M13	62°/58°	400	CCACAACCTTCCTGCTAACC	CCAACAAAAACACACACACG
Abur139	5	FAM-M13	62°/58°	336	TCTCGCAGGCTCTAATCAGC	TACTGCTCACCGCTTCACC
Abur140	21	HEX	58°	200	TGCCGAGAAACAAAGTGATAG	CTGGAGCTGTGCCTCGTAAG
Abur141	7	FAM-M13	62°/58°	278	AAGGGAATAAGCGTCGAAGG	ACCGTCATAATGCGGCTATC
Abur144	2	FAM	55°	332	TCCCTGTTCAGGAAAGAACC	TAAAGACGTCCGGACAGGAG
Abur145	22	FAM	55°	127	GCGGTGTTTAAGCTCGCTAC	TTCTGCCTGTAATCACACCATC
Abur146	12	FAM	55°	230	TCATGCCATTTCTGCTTGAG	CGATATGCACTCCACAGACG
Abur147	unassigned	HEX	55°	201	TTGGCACCTTGAGTGTTCAG	TGTGACACTCGCTCAAAGATG
Abur148	22	FAM-M13	58°/53°	179	CACTGTGCGAAAAGTGCTTG	AAGCCGTCAGACTCCTGAAC
Abur149	12	HEX	55°	311	AGGGCAAACCAAAGCTGAG	GGAAGCCAGGGTAAGTCCTC
Abur150	4	FAM-M13	62°/58°	380	CACAGCCACACAATGATGTC	GCAGCTGCTGTAGAGTCGTG
Abur151	6	NED	58°	249	GTGAATGTGTGAGCGTGTCC	CAGATGCAAACAGCTCGAAG
Abur161	3	NED	55°	150	GCATGTTCACAACTCACTAGCC	GCTGGAGAGGTCATTCTGAGG
Abur162	4	NED	58°	210	CGACCGTTAGTATTCCTGCTG	CTGAGGGATGCCAGATGAG
Abur163	7	NED	58°	364	TGCAGACTTTGCTTGAGTGC	TCAACTGCAATTCCTGTGAGTAG
Abur164	15	NED	58°	425	TGCACCCTTCAGACAAACAC	GCCACTTGTGAAATGAACATAAC
Abur165	25	NED	58°	330	TTTATGATGCCAGTAGTGATGC	CGTCTAGTCCGATGCCAAAG
Abur167	24	FAM-M13	58°/53°	385	GCGCGCAAGCAGTGTCTC	CCTCCTCCTCGTCTCCTTCT
Abur171	1	NED	55°	164	GGAGACCTGCAAGTCAATCC	TTGATTGCAACGTATTAACTGATAAC
Abur174	2	NED	58°	342	TGAGGCCAGACCTACCAAAC	TCTCCATCGCTCCTCACAG
Abur176	14	NED	58°	388	AATGGAGCTCGCTGGTAGTC	CTGCACCTGCTGTATGCAAG
Abur178	2	NED	58°	152	GGTTTGAAGAGAGCGAGCTG	CCGGTCAGTGTACTCACAGTATC
Abur179	2	FAM-M13	58°/53°	250	AGTCTTCATTTGCCCTGCTG	TCTCTCACCATGTCGGTCTG
Abur182	17	FAM-M13	62°/58°	150	ACCCTGAGAGGGAGGGATAG	AGGAGGCTGCTAACAGATGC
Abur183	8	FAM-M13	62°/58°	334	TGCACTTGGAGCCAGATAAG	GAACATTTCTCAGGGATTTCG
Abur184	13	FAM-M13	62°/58°	211	CACTCTTGGGGGCTGTAATG	ATGCCGAATCTTTGGAAATG
Abur187	11	FAM-M13	62°/58°	282	GTTTGGTGTGTGGGAGTGC	TGTCAAAGCTGGATTGATGG

Abur189	16	FAM-M13	62°/58°	424	GCTTCATGTATAGCAGACATCAGTG	TGTGAAAATCAAGTTTTGTGCTC
Abur190	unassigned	FAM-M13	62°/58°	274	TAAAGTCCGTTTGGCTCGAC	GGGGAACCACACTAGAAATCC
Abur191	18	FAM-M13	62°/58°	242	ACTCACCGCCACTCATCTTG	AGCAGTCACACCGCTAGAAAC
Abur193	14	FAM-M13	62°/58°	219	GGTAACGCAGGAAGTCAAGC	AAAACCGCAATTTGAGTCTG
Abur194	7	FAM-M13	62°/58°	154	AGACCCTGATGGTGGAATTG	CCCAGCTGCTCAACTCTCAG
Abur196	22	FAM-M13	62°/58°	373	GACGCAGTTTCAATGTACGG	CCTGTGGTTAGCAAGGTCTG
Abur197	4	FAM-M13	62°/58°	131	GACAGGGACACAGAGGAAGG	CTTCTCAATCCCACCCGTTC
Abur199	20	FAM-M13	62°/58°	446	GACTCAACGAAACCGAGCAG	GCCTCCTCCAGAGTCTCACC
Abur202	19	FAM-M13	62°/58°	243	CACCTTGGCATTTTCATGTG	CCATTTCCTCTCCAGCTCAG
Abur203	15	FAM-M13	62°/58°	170	TGCAGCAGAGGTGAGACTTG	TGGTCATCCAGAGGAAGGAG
Abur204	5	FAM-M13	62°/58°	268	CTGCACCCAGGAAAAACAAC	CTTTTAGCAGCCCTGTGACC
Abur205	3	FAM-M13	62°/58°	219	TCTAATAAGAATATTACCCATCATGC	GTGCTGGGATTTCAGTCCAC
Abur206	23	FAM-M13	62°/58°	299	GGTCTGATGGCTCTCTGCTC	ACACAGCTGCACGCACAT
Abur207	17	FAM-M13	62°/58°	143	ACCCTGAGAGGGAGGGATAG	AGGAGGCTGCTAACAGATGC
Abur208	13	FAM-M13	62°/58°	353	GGAGAAGGAGGAGCAGCAG	ATGCCGAATCTTTGGAAATG
Abur209	8	FAM-M13	62°/58°	138	CTTGGTGGTGTTCGTTACCC	AGATGCAAGCCATCACTTTG
Abur212	8	FAM-M13	62°/58°	249	AGAGGCCAGCAGCTCATTC	GCCCCAGGTTCCACTAAGG
Abur218	6	FAM-M13	62°/58°	133	CACAATAACAGTGCTGGGTCAG	CGGTCTGCAGAGACATGGAG
Abur221	1	FAM-M13	62°/58°	125	CAACAACAGGCAGTCGAAGG	CTCGGCATGCAGGTTTCC
Abur223	8	FAM-M13	62°/58°	179	TGAGAACTTGGGGACAAAGC	GGAAGCACAGCTGAGAAAGG
Abur224	16	FAM-M13	62°/58°	312	GAGCGGTGTATGTGTGACG	TGCAGTGCTGTAACTGAACG
Abur225	1	FAM-M13	62°/58°	246	TCTTACAAAATCCATTCCATGC	TGAAAGAGCAAGTTACGAAGACC
Abur226	6	FAM-M13	62°/58°	226	TCACTTCAACAACCCACAGC	TAATGCTTCACCGACTTTGC
Abur227	18	FAM-M13	62°/58°	290	CAGCAGAGCATCAATTCACC	AATCTGCAAAACTGGATCTGG
Abur228	1	FAM-M13	62°/58°	155	TTTGTGGCTGTTTGTGAAGG	GTGATTAAGGATAAGTGAGTGTGC
Abur230	1	FAM-M13	62°/58°	294	TTGAGCTCTTATCCCACTTGC	ACTGAGAATGGACAGCATGG
Abur233	7	FAM-M13	62°/58°	300	TGATTTGCAAACTTGGATCG	GGAGCAGGAAATTGCAACC
Abur234	11	FAM-M13	62°/58°	198	CCACTGGGAACTGAAACTGC	GGCGATCCAGAGTGTGACC
Abur235	13	FAM-M13	62°/58°	273	GAATCTGCACAATGCCAAAC	CAGTACTGAGGCCACCTTTG
Abur239	9	FAM-M13	62°/58°	325	CACATTATTTCTACCCCTAACACC	TCCCTCAGCTAATCCCTTACC
Abur240	3	FAM-M13	62°/58°	188	TCATTACAATAACACCAACTGTGC	TTTATGGTGGGAAAGGCTTG
Abur242	2	FAM-M13	62°/58°	266	TCTTGCAAGCCTGTTTCTCC	GGTTTTATCAACCCGAGTTCC
Abur244	18	FAM-M13	62°/58°	172	CCACATGACAGGGACAGAGG	CATGGTTAGGGCATCTGTGG
Abur246	10	FAM-M13	62°/58°	184	CAATGTTCCTCTTCACCAACG	CACGTTTCGAGTGAAAATCG

Abur247	1	FAM-M13	62°/58°	319	GCTGCTTAGCATCAACAAGC	AACGCTTCACTCAAACAGTAGC
Abur248	6	FAM-M13	62°/58°	479	CCACCATCACATTATGTAAGAGACC	ACTCACCGCCCACACTCAC
Abur249	3	FAM-M13	62°/58°	327	TCAGCTGAAAAACAAAAGACG	TTTTGTTATGACGAGTGGAAGC
Abur251	9	FAM-M13	62°/58°	349	TCGGGTAATTATGGGAGAGG	GAACTCTGAAAATCAAGGTCACG
Abur254	22	FAM-M13	62°/58°	302	ACACAGAAGCAGCCACAGC	AAACCGCAGATTCGTGTACC
Abur266	7	FAM-M13	62°/58°	200	AAACCAGTCCAGCTGTTTGC	CTGTGATTGGACGGTTTGTG
Abur276	23	FAM-M13	62°/58°	243	TTGGAACTGTTTCAGTGAACG	AGACAACGACTGCCACGAC
Abur277	13	FAM-M13	62°/58°	327	CCCCTTAAACCTCAGCAGTG	GTCTCGAGTCTGTGCAGTGG
HoxAa	15	FAM-M13	62°/58°	273	TGTCTTTGAAGCGTGGTCTG	ATGGAAACGTCCAACTTTGC
HoxAb	4	FAM-M13	62°/58°	280	CCAGTGGAAAAGAAGGTTGC	CCATGGGCTCCAATATGATG
HoxBa	16	FAM-M13	62°/58°	338	TCCTGGGAAATAGGGAGAGC	ATTGTTCCAAGCCCCAGAC
HoxBb	20	FAM-M13	62°/58°	324	TTATTTGCCCCTCTGGACTG	CTGCCTCTGTCCTCCTTCTG
HoxCa	11	FAM-M13	62°/58°	337	TCCTGCATTCCAGTCTCTCC	AACCATTTCTGCTGTCGTCAC
HoxDa	22	FAM-M13	62°/58°	158	CAAAGCCCCAGACAAAAATC	CCGGTCTACACAAAAATACGC
HoxDb	14	FAM-M13	62°/58°	226	CATCTCCGGTGGCAAGTAAG	ACTGCATGGGGACATCATTC
ParaHoxC1	18	FAM-M13	62°/58°	287	CATAGTCTATTCCACCACCAAGG	TTGATAAAACTTGGATATTACAAGAGC
Ppun1	12	FAM	55°	191	GCTCATATTGGAGGGCTGAG	CAGGGATATTTTTGCAGTGAAG
Ppun5	8	FAM	55°	Taylor *et al.* 2002	TGTTTGTGAGTCCTTTTGTATCG	GCCCATATAATACCAATGTGCAG
Ppun6	14	HEX	55°	255	CTGGCATACAGTATCAGAAAA	ATAGTCCAATGAAGTGTGGA
Ppun7	2	FAM	55°	Taylor *et al.* 2002	TGACCATCTGCGACAAATAAC	AGGCCTAAGTCCCCCTAACC
Ppun12	4	HEX	55°	277	TGGGCAAAGTAAACATGCAC	TCTGTTAGCTGGGAGGCAAG
Ppun16	12	FAM	55°	324	CCAGGGTGTTGTATGTGAAGG	TGCTGTTGTGAAGGTTTTGC
Ppun18	6	HEX	55°/50°	254	CCGCACTTGTTTATCTTCACAC	CATGCAAACCCAACCTCAAC
Ppun19	9	FAM	58°/53°	204	CATGGTTTTGTTTGCCGTTC	TCTAGCATCAACAGCCATGC
Ppun20	2	FAM	58°/53°	Taylor *et al.* 2002	ATTGCCCATTTTCAGAAAGC	TGGACATTTTCAGAGTAAGGAGAG
Ppun24	8	HEX	55°	297	TGGGACCTTCTTGCTTTTTG	GCGGAGTTCTGCTGGTATTC
Ppun34	3	HEX	55°	134	TTGCCTAATAATTCTGCACTCC	TGCATGCCTCATACACACAG
Ppun35	3	FAM-M13	58°/53°	347	TCCCAATTTCAATAACACAGG	GCAGATTGCAGCTGAGAAAAG
Ppun41	14	FAM-M13	58°/53°	119	TCACCATCAGAGCTGCTCAC	AACCTCATCTTGCTTTCAGTCC
TmoM5	10	HEX	55°	Zardoya *et al.* 1996	GCTCAATATTCTCAGCTGACGCA	AGAACAGCGCTGGCTATGAAAAGGT
TmoM7	11	FAM	50°	Zardoya *et al.* 1996	CTGCAGCCTCGCTCACCACGTAT	CACCAGATAACTGCACAGCCCAG
TmoM27	12	FAM	48°	Zardoya *et al.* 1996	AGGCAGGCAATTACCTTGATGTT	AGCTCGGCGAATGATGAACTCTT
OSU9D	5	FAM	50°	Wu *et al.* 1999	CCTCTGTAGTGATGTTTAATCTCTGT	TGACACTGCACTTACTTGGCT
OSU13D	16	HEX	50°	Wu *et al.* 1999	TAAGCTGATAGGAACCCAAC	ACTCCTATTTTGTTATTTTTGTGA

OSU19T	12	FAM	50°	Wu *et al.* 1999	TGAAGGACAAAGCAGGACTG	TGCCCGAACCTTTTTATTTA
OSU20D	16	HEX	50°	Wu *et al.* 1999	GAAGTGGGATTTGCAGCTTG	CATGCTTACAAAGAACAGGGTTAC
UNH002	unassigned	FAM	55°	Kellog *et al.* 1995	TTATCCCAACTTGCAACTCTATTT	TCCATTTCCTGATCTAACGACAAG
UNH106	19	FAM-M13	55°	Kocher *et al.* 1998	CCTTCAGCATCCGTATAT	GTCTCTTTCTCTCTGTCACAAG
UNH130	18	NED	55°	Kocher *et al.* 1998	AGGAAGAATAGCATGTAGCAAGTA	GTGTGATAAATAAAGAGGCAGAAA
UNH152	8	FAM-M13	55°	Kocher *et al.* 1998	TCATCAGAATCATGTTTATTG	GTATGTATGTGTATGCATGTGTAA
UNH192	4	FAM-M13	55°	Kocher *et al.* 1998	GGAAATCCATAAGATCAGTTA	CTTTTTCAGGATTTACTGCTAAG
UNH2004	9	FAM-M13	55°/50°	Albertson *et al.* 2003	ACAAAAGGTTACTGCACTGAGAG	TGGTTTATCTCACCGCTTCA
UNH2005	9	FAM-M13	55°/50°	Albertson *et al.* 2003	AGTCAAGCAACACAGGGTGA	TCACTTGAATCTCTTCTTTTAGTTGG
UNH2008	8	FAM-M13	55°/50°	Albertson *et al.* 2003	CATGGACCAGTGGAGAACCT	GCATCCTGCTTTTTCCTTTG
UNH2032	1	FAM-M13	55°/50°	Albertson *et al.* 2003	ACTCGGGCTGTCTGAGAAGT	TGTTTCCCTCTGAACGGAAG
UNH2037	21	FAM-M13	55°/50°	Albertson *et al.* 2003	GGGATTCACTGGCACCTACT	ATGTGGTTCCCAGTGATGGT
UNH2044	1	FAM-M13	55°/50°	Albertson *et al.* 2003	CTGCTGTCGGGGAAATTTTA	GGAAAGAATTGTGGCAGAGG
UNH2046	23	FAM-M13	55°/50°	Albertson *et al.* 2003	TTCATCAACATCCTCACCAGA	ACGACAGAAGAACCTGCACA
UNH2056	18	FAM-M13	55°/50°	Albertson *et al.* 2003	CTTTCCTCGCAAAAATGAGG	TGACAGACATGGCTCTTGAGAT
UNH2058	8	FAM-M13	55°/50°	Albertson *et al.* 2003	TTTGTTCTGCAGCTTACCTCA	TGGCAGCAGAAGTAGCATTG
UNH2059	21	FAM-M13	55°/50°	Albertson *et al.* 2003	TTGGAGCTGCTTTCTTACCC	TGAGCTTCATTATTCAAACAAACC
UNH2069	20	FAM-M13	55°/50°	Albertson *et al.* 2003	GGGGAGGTAGAGGCCATTAT	GTCCACCCACGTCCTGTATG
UNH2071	8	FAM-M13	55°/50°	Albertson *et al.* 2003	AATCTCCTGCTCTTGCTCTTT	GGCCTCCTGCATAGTAAACA
UNH2075	unassigned	FAM-M13	55°/50°	Albertson *et al.* 2003	AGCTGAGAGGGCTTGTGAAG	AGAGCCGCTGTTAAGGGATT
UNH2080	21	FAM-M13	55°/50°	Albertson *et al.* 2003	CCTCATTCCTCTCTGGGACA	ATGGCATCTCCCTTTTCACA
UNH2084	5	FAM-M13	55°/50°	Albertson *et al.* 2003	TCCATCCAAGGGTCAACTTT	TGAGGTGCATATTGCTGCAT
UNH2094	7	FAM-M13	55°/50°	Albertson *et al.* 2003	ACAGTTTGGGTCAGTGGTCA	TGATATGTAGTGAGCAAAACCAA
UNH2100	7	FAM-M13	55°/50°	Albertson *et al.* 2003	TTTGTCATGCCCTTTCAGTG	CGGTCAATACCATGTCACTCAG
UNH2104	12	FAM-M13	55°/50°	Albertson *et al.* 2003	CCCTACAGGCGCTAACACAT	TGCTCTATTTCCTGTCCATCC
UNH2112	15	FAM-M13	55°/50°	Albertson *et al.* 2003	CTCGGTGGTCAGAATGAAGG	TTACAGCACTTCACGGTTGC
UNH2116	1	FAM-M13	55°/50°	Albertson *et al.* 2003	CACAGACCCACAGACGTTTC	GGAGTCTCTTTGCCTGAGTGA
UNH2117	1	FAM-M13	55°/50°	Albertson *et al.* 2003	TGTTTGCCGTTATCATCCAC	CTTTGACGTGTTTTGCAGGA
UNH2125	18	FAM-M13	55°/50°	Albertson *et al.* 2003	CCAATCCATTGCAAACACTG	CTTTCCTCGCAAAAATGAGG
UNH2134	9	FAM-M13	55°/50°	Albertson *et al.* 2003	TGCAGTGCCGACACACTTA	GCTCTTCAGGCCAGGTGTAG
UNH2139	3	FAM-M13	55°/50°	Albertson *et al.* 2003	GCAGTGCACATGCGACTTAT	ACAGCCAGCTACTGTGCAAC
UNH2141	12	FAM-M13	55°/50°	Albertson *et al.* 2003	CACCAACTAAAATTTTGCCAAC	GCTGATTTTTGTTTTCCGTCA
UNH2149	2	FAM-M13	55°/50°	Albertson *et al.* 2003	CATTTTGCATCGTCAGGAGA	TCAGGCATTGCAGATGAAAC
UNH2150	2	FAM-M13	55°/50°	Albertson *et al.* 2003	TCAAAGAGCAAGAAAAGGGTAAA	GCGCCCCTTCCTTCTACTAA

UNH2153	1	FAM-M13	55°/50°	Albertson *et al.* 2003	TGAAGAGAATGTGTGGAACGTC	ACAGATGCCCACTTTTCTGG
UNH2163	18	FAM-M13	55°/50°	Albertson *et al.* 2003	CCTTGTGTTTTTCTCTGCTCAA	CCAAACGAGGAGGATCGTTA
UNH2166	2	FAM-M13	55°/50°	Albertson *et al.* 2003	ACTGGCCCAAAACTGTCAAA	TGTGTGCCAAGGATAGCAAA
UNH2169	6	FAM-M13	55°/50°	Albertson *et al.* 2003	CCAGTGGGTCCTCCTACAGA	CCCAGTGACTTTGAGGTGTG
UNH2181	unassigned	FAM-M13	55°/50°	Albertson *et al.* 2003	GACCTCAGGACAAAGCAGGA	GATCACTCGCAGCTAGGACA
UNH2185	1	FAM-M13	55°/50°	Albertson *et al.* 2003	TGGAGAAGAAAACCCAGTGTG	ATGACAGCACGCATTGTTTC
UNH2191	17	FAM-M13	55°/50°	Albertson *et al.* 2003	ACCACGCTTGTGTGAATGAG	AAACCCAAGCTCTGACTCCA
UNH2204	10	FAM-M13	55°/50°	Albertson *et al.* 2003	CACATCATGTCAATCAGACATCC	GGAGACGGTTCAAAGTCCTG

TABLE S2

Inferred linkage group synonymies among the *A. burtoni* map and the other two maps of East African cichlids, *M. zebra/L. fuelleborni* and *Oreochromis* spp.

Linkage Group		
A. burtoni	*M. zebra/L. fuelleborni*	*Oreochromis sp*
1	1	7
2	16	15
3	12	-
4	-	11
5	3	-
6	11	-
7	5	6
8	4	-
9	6	1
10	8	-
11	-	-
12	3	-
13	-	-
14	-	-
15	13	22
16	-	-
17	7	9
18	10	23
19	-	3
20	20	-
21	2	19
22	-	-
23	18	-
24	-	-
25	-	-

No entry (–) indicates unknown relationship of linkage groups.