Supporting Information

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SI Materials and Methods

Pixel Density Analysis (PDA). To determine and compare the relative amounts of *Ptch1* transcript within the region of the pharyngeal skeleton that contains the retroarticular process, PDA was performed as described previously (1, 2). In brief, after whole-mount in situ hybridization (WISH), specimens were mounted in 80% glycerol and photographed with a Zeiss Axiocam digital imaging system mounted to an M2 Bio stereomicroscope (Zeiss). Homologous areas within the jaw were selected using the lasso tool in Adobe Photoshop, and the total number of pixels within that region was determined using the histogram tool (Fig. S4). Next, the relative area in which *Ptch1* was expressed was measured by selecting for pixels that were the color of the in situ reaction (i.e., purple–dark purple) using the "select color range" option within Photoshop. For *Ptch1* and *Gli1* expression this analysis was performed on the area ventral to the eye (as shown in Fig. S4). Because *Col1a1* expression is more restrictive, PDA was performed on the region immediately adjacent to the RA (i.e., Fig. 3*D*, main text). Finally, as a point of comparison, similar analyses were performed using all three probes on the caudal fin. (GenBank accession nos.: *Gli1*, JN037689; *Ptch1*, JN0376990 and JN037691; *Rad26l*, JN037692 and JN037693; *Col1a1*, JN116727).

Quantitative PCR Analysis. Larval fish were staged as described in the main text, and tissue microdissected at 6.5 days postfertilization (dpf) and preserved in RNAlater (Ambion). Lower jaw cartilage back to and including the first four ventral pharyngeal arch tissues (i.e., the mandibular, hyoid, and branchial arches 1 and 2) were taken, as were the tail and trunk posterior of the yolk sac. RNA was isolated using the RNeasy kit (Qiagen) and quantified by absorbance. Normalized RNA was used as template for reverse transcription by SuperScript III (Invitrogen). Primer-probe sets were designed across exon junctions using Primer Express (Applied Biosystems) software (*Ptch1*: forward, 5′-TTCTGATGCTGGCCTATGCA-3′; reverse, 5′-CCCCTGAGACTTGGCACAGT-3′, probe, 5′-6FAM-CCTGACCATGCTGCGAT-TAMRA-3′; *Rad26l*: forward, 5′-CGAGTCCAGATCGTCAGAGACTT-3′; reverse, 5′-CACCTGCCATGGTGGAAAC-3′, probe, 5′-6FAM-AACAGCTCCTCTCACATC-TAMRA-3′). Real-time PCR reactions used Taqman Universal PCR Master Mix (Applied Biosystems) and were run on a Roche Lightcycler 480. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method (3), normalizing to β-actin expression as an endogenous control.

1. Albertson RC, Yelick PC (2007) Fgf8 haploinsufficiency results in distinct craniofacial defects in adult zebrafish. *Dev Biol* 306:505–515.
2. Cooper WJ, Albertson RC (2008) Quantification and variation in experimental studies of morphogenesis. *Dev Biol* 321:295–302.
3. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{(-\Delta\Delta C(T))}$ Method. *Methods* 25:402–408.

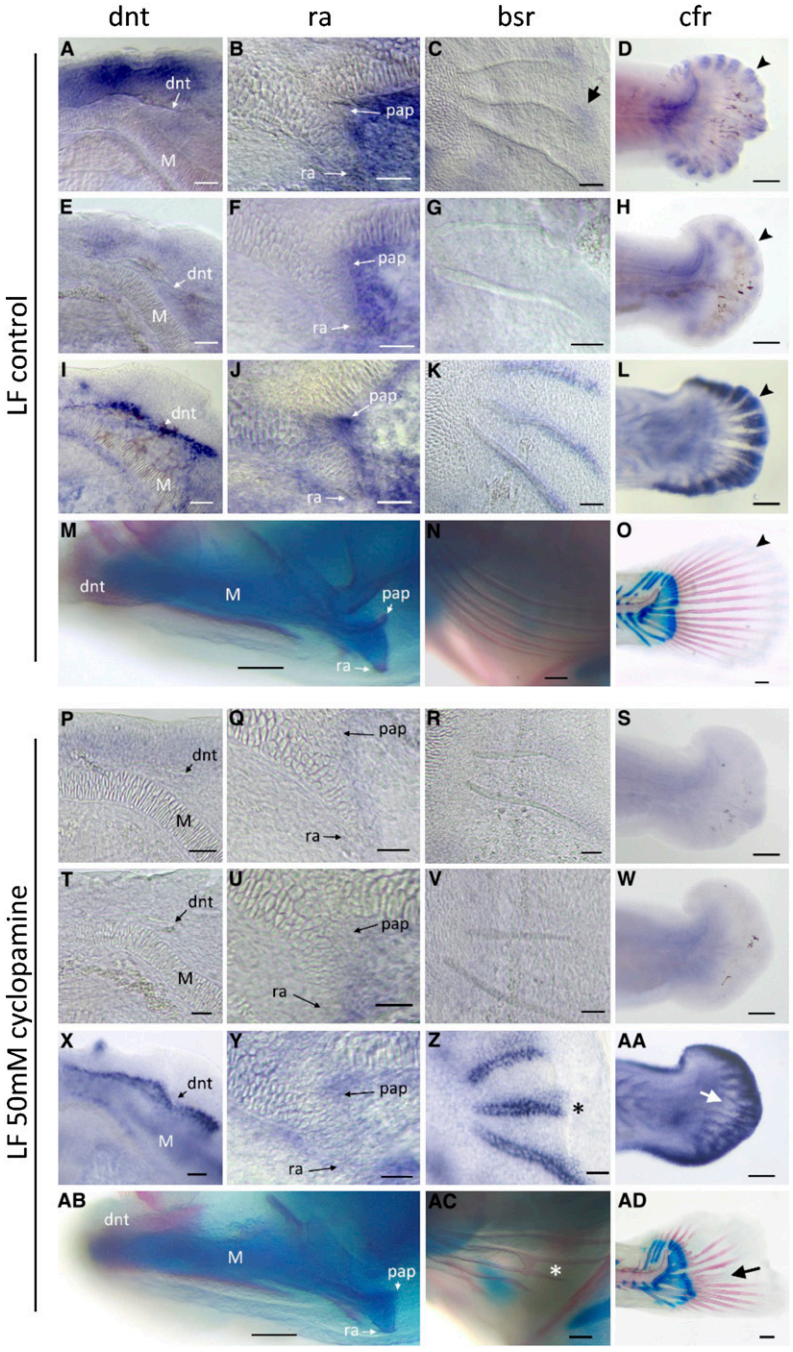

Fig. S1. Hedgehog pathway is necessary for proper craniofacial bone development. Expression of the hedgehog receptor *Ptch1* (*A–D*), its downstream target *Gli1* (*E–H*), and the bone differentiation marker *Col1a1* (*I–L*) is shown in 6-dpf (stage 12) *Labeotropheus fuelleborni* (LF) larvae. Colocalized expression was observed around the developing dentary (*A, E, I*), retroarticular (*B, F, J*), branchiostegal rays (*C, G, K*; note the discrete node of *Ptch1* expression at the distal end of these bones in *C*, arrow; although *Gli1* expression was not observed around these structures, *G*), and within the dermal fin ray elements of the caudal fin (arrowheads *D, H,* and *L*). Mineralized structures are shown as a reference in older fish (12 dpf, *M–O*). Treatment with a hedgehog pathway inhibitor resulted in the down-regulation of hedgehog signaling and aberrant craniofacial bone development. LF larvae were treated with 50 μM of cyclopamine at 5.5 dpf (stage 11) for 6 h. Expression of *Ptch1* (*P-S*) and its downstream target *Gli1* (*T–W*) were drastically reduced. Although expression of the osteoblast differentiation marker *Col1a1* was relatively unaffected in the dentary (*X*), its expression in other structures suggests an attenuation and/or delay in bone development. Specifically, *Col1a1* expression was reduced around the retroarticular process (*Y*), whereas expanded expression was observed around the branchiostegal rays (*Z*) and within the caudal fin (*AA*), suggesting that these structures are in a more undifferentiated state relative to control animals. We also noticed bifurcated expression of *Col1a1* in the branchiostegal rays of cyclopamine-treated animals (asterisk in *Z*), as well as disorganized expression within the developing caudal fin ray elements (arrow in *AA*). The phenotypic outcome of this treatment is consistent with altered patterns of gene expression. Although the dentary is relatively unaffected in cyclopamine-treated animals, the length of the retroarticular process is reduced in treated animals (*AB*). In addition, the branchiostegal rays are bifurcated and fused in treated animal (*AC*), and dermal fin ray elements are dramatically reduced in the caudal fin (*AD*). bsr, branchiostegal rays; cfr, caudal fin rays; dnt, dentary; M, Meckel's cartilage; pap, posterior articulation process; ra, retroarticular. (Scale bars, 10 μm in *A–C, E–G, I–K, P–R, T–V,* and *X–Z*; and 100 μm in *D, H, L–O, S, W,* and *AA–AD*.)

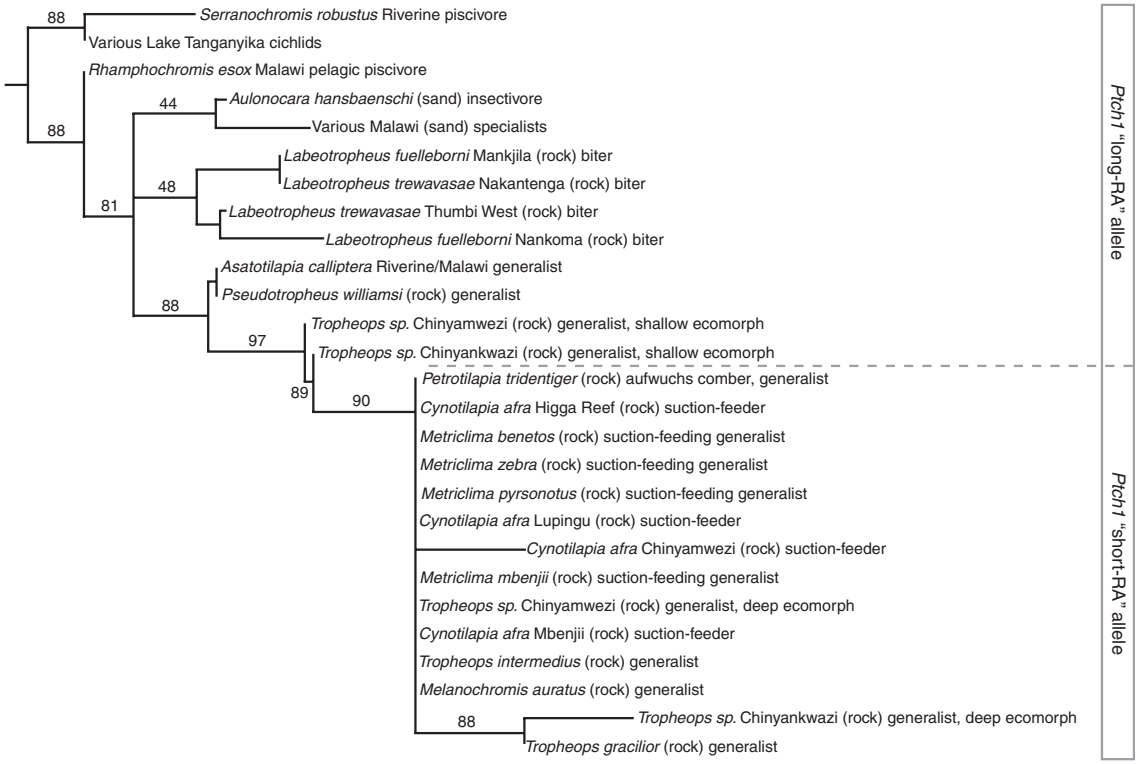

Fig. S2. *Ptch1* gene tree. Neighbor-joining tree of representative *Ptch1* locus genotypes by species and site, based on 32 polymorphic sites. Haplotypes with the "short" *Ptch1* allele SNP genotype at the high MA_O association SNP (Fig. 1*F*, main text) form a single derived clade found in Malawi rock-dwelling cichlids. The gene tree can be contrasted to previously published species phylogenies of the three genera analyzed in the main text (1) and East African cichlids in general (2). Gene tree created with MAFFT (3).

1. Albertson RC, Markert JA, Danley PD, Kocher TD (1999) Phylogeny of a rapidly evolving clade: The cichlid fishes of Lake Malawi, East Africa. *Proc Natl Acad Sci USA* 96:5107–5110.
2. O'Quin KE, Hofmann CM, Hofmann HA, Carleton KL (2010) Parallel evolution of opsin gene expression in African cichlid fishes. *Mol Biol Evol* 27:2839–2854.
3. Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30:3059–3066.

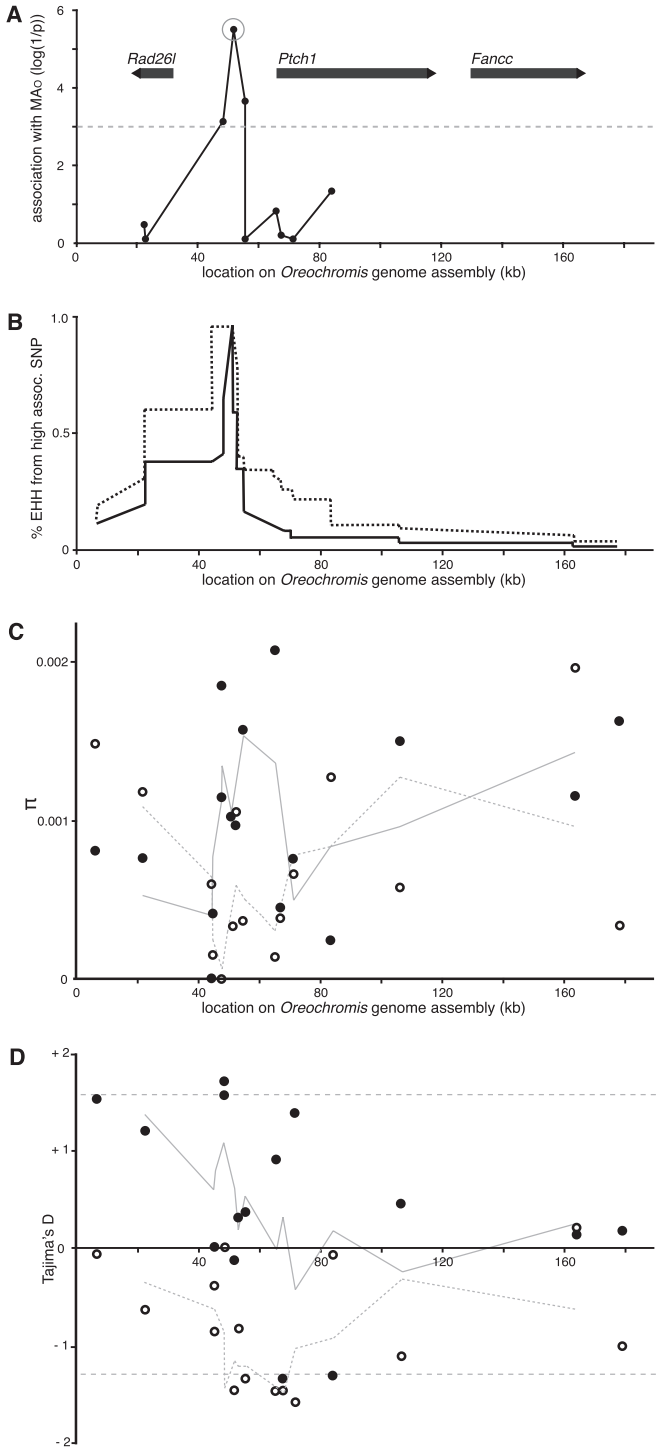

Fig. S3. Signatures of selection upstream of the *Ptch1* gene. (*A*) Association between SNP genotype and the quantitative phenotype MA_O in natural *Tropheops* populations, as reference for *B*–*D*; all share the same *x* axis for comparison. (*B*) Extended haplotype homozygosity (EHH) from the high MA_O association SNP. *Ptch1* short alleles (dashed line) show greater relative EHH than long alleles (solid line). Genotypes were phased in FastPhase (1) and the EHH plot made in Sweep (2). (*C*) Nucleotide diversity (π) and (*D*) Tajima's *D* values for comparison of *Labeotropheus* (closed circles, solid lines) and *Metriaclima* (open circles, dashed lines), calculated in DnaSP (3). Lines indicate sliding average of three adjacent values. *Metriaclima* show consistently reduced π in the region surrounding the high MA_O association SNP, as well as consistently low Tajima's *D*, indicative of recent positive selection. Dotted horizontal lines in *D* indicate significance thresholds determined by coalescent simulations in DnaSP (3).

1. Scheet P, Stephens M (2006) A fast and flexible statistical model for large-scale population genotype data: Applications to inferring missing genotypes and haplotypic phase. *Am J Hum Genet* 78:629–644.
2. Sabeti PC, et al. (2002) Detecting recent positive selection in the human genome from haplotype structure. *Nature* 419:832–837.
3. Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.

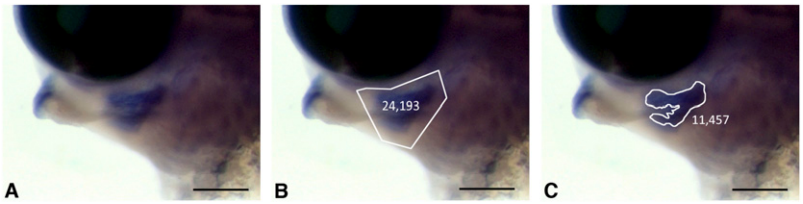

Fig. S4. Pixel density analysis of *Ptch1* WISH results. After WISH, specimens were mounted in 80% glycerol and photographed (*A*). (*B*) Homologous areas within the jaw were selected using the lasso tool in Adobe Photoshop, and (*C*) the "select color range" option within Photoshop was used to select pixels that were the color of the in situ reaction. (Scale bar, 200 μm.)

Table S1. *Ptch1* locus markers

Amplimer*	Primer 1	Primer 2	S†	Loc‡	Marker	Sequence flanking polymorphism	S_{ML}§	S_T¶
Ptch1Loc1	CCACTTAATTGCCTCGTCTCA	TTCTCGGCTACAAAGCCACT	5	179.1	Loc1_SNP1	AGAAAAAAAGAGAGAGAGGGGGAAC(A/G)GGTCAGTGAGCATTTTCTGATGGGC	+	
					Loc1_SNP2	GAGCATTTTCTGATGGGCTCAGGAT(C/G)GTCTCTACTCGATGTGGAGGCATTT	+	
Ptch1Loc2	ACTGGGTACAGGTGGCTGTC	TTGGAGGAGATGGTGGAGTC	7	164.4	Loc2_SNP1	TGAGATGGTGATCCCTT(C/T)CTGTGGCTGTTGATATTCTACCACT	+	
					Loc2_SNP2	ACAGAGCTTCACAGCTGGTGAGTTT(G/T)TTTGTTTTTTGCATAAATCATTTTA	+	
					Loc2_SNP3	TTATTTGCAGGTCTGTTGTTGTTGA(A/C)AGCAAAAGAAACAATACAGAGGGGC	+	
					Loc2_SNP4	TTTACCTCTCTGTCTCTTGCTTGTC(C/T)GCCTCCCATCATAACCCTTGGAGAC	+	
Ptch1Loc3	CAATCCCGGAGCTTTTTACA	TGCTAGAGCCCCATGGTAGT	6	106.5	Loc3_SNP1	AGCTCAGTCATGCACAAATGCACAC(A/G)TATTGCAGTGGTACAGTAAAGTCTA	+	
					Loc3_SNP2	GTATGTTTGGCTGTACCTTACAGTA(A/G)CATTTCAAATTAATGTGGAGCTGGA	+	
Ptch1Loc4	GGATCATCAGCTGAGGGGTA	CTGCGATGTCTGTGGGTCT	6	84.1	Loc4_SNP1	AGTCTGTGTTTTTCTGACACAAAGG(A/C)ACAAATATCTGCAAAATATAGACAC	+	+
Ptch1Loc5	TCGTGTGTGGGGAGACAGTA	TGCCTGTTTATGTTTCATTTGG	5	71.4	Loc5_SNP1	TACAATCTGCCCTAAATAGAAACCA(C/T)GGTCACTTTGATGTTTTCTAAGTGG	+	
					Loc5_SNP2	TCAGAAAGCAGGTGACGACCGACAC(A/G)ACACGAGATCGCAAACGTGAATGTT	+	
					Loc5_SNP3	GCTGAGAGATCTGATGGGAGCTGGG(A/C)TGTATTGTATGAGCGCACGCATCCA		+
Ptch1Loc6	GTAACCTGCACGCACACATC	AAAATGTGGCCAAACTGTCC	5	67.4	Loc6_SNP1‖	CTGCGAGAGTAAAAGCTGCATGTTT(A/T)TTGCCTGGTGCGGGACTGCTCTAGG	+	+
Ptch1Loc7	CGTGATTGGCACAGTTTACG	TTGCTGCACAATAAGCAAGC	5	65.7	Loc7_SNP1	CCAAGAGTGGCTGCTGGAGTTAATT(G/T)TTTTGCTTATTATAACCTTATTATT	+	+
					Loc7_SNP2	TCAGAATAAAGTCAATTATAAAAAT(G/T)ATGCCTGCAGCACGGATAGATAAAT	+	
Ptch1Loc8	GCACACTAGACACTTAACCA AAACA	GCATCAGCTGTGCTGGAAT	5	55.3	Loc8_INDEL1	TATATGTCCCGGATTTAACCGTGTT(-/TGGGCCGCTT)AAGCTATGACTTCATGGTATTGTG	+	
					Loc8_SNP2	TGGGCCGCTTAAGCTATGACTTCAT(A/G)GTATTGTGAAGAAAATAAGCAGACG	+	+
					Loc8_SNP3	AGAAATGTGACTTGAAAAAGAGCAG(C/T)GAATGAGAAAATTCCCTTGTTGTGT	+	
					Loc8_SNP4	TTTTTTCTTAATGTGTTCTGATGGT(C/T)AAAACAATCCAGAATCAAATGTGGC		+
Ptch1Loc9	GGCCTTACTGTCGTCGTTTC	ATAGGGTTAGGCTCGGGTTG	5	52.8	Loc9_SNP1	TGTCTTCCAAGCTACTAAAACTCCG(C/T)ACATTATTACAAAATATGGTGCCCT	+	
					Loc9_SNP2	GGGGGATTCGGAGTGTGTGTGTTTG(A/G)AATGTTTGTGAAGCCAGTCTCTGAT	+	
Ptch1Loc10	CAATGGTTTTAAACACAAC TCTGG	TCATCCGATCATCCACTTGA	6	51.8	Loc10_SNP1**	TTCCAGAAATACTAGGAAAAACACA(C/T)GCAGTAGTTACTAATGCCTTGAATG	+	+
					Loc10_SNP2	ATGAGTAATGGCAAATTTAAAAAAA(A/C)CCTCACTGTAGTATGTTTGTTTTAC	+	
Ptch1Loc11	AGCATCTGGTTCAGGGTCAT	CAGGGCTGAAGATGTGACAA	1	48.3	Loc11_SNP1	ACACTGCTCACAAAGACTCTAGTCT(A/G)GTGTCTTTGTCACATCTTCCAGCCC	+	
Ptch1Loc12	CCCCAGGAAATAGATAGAC TCC	CACAGTGCACACTGCTCACA	3	48.4	Loc12_SNP1	GGATTTAACAAGTGCATGTAACAGC(C/T)TCACAGCCATGCCTTTTATGGCCTT	+	+
					Loc12_SNP2	CTTGTAAATTATCATAATCTAAAAC(A/C)AGTTTGCTCACTACGTCAGGTGGCG	+	
					Loc12_SNP3	AGGGCTGAAGATGTGACAAAGACAC(C/T)AGACTAGAGTCTTTGTGAGCAGTGT	+	
Ptch1Loc13	TTTGTCTGTTTTGCACATCCTC	CCGTTCTTCCTGGGTGAAT	1	45.3	Loc13_SNP1	AGACGGTATGAAGGGAGGAGAGGAA(A/G)GAGAAAAAGAAAGAGAGTCATGGAG	+	
Ptch1Loc14	CTCCTTTCATCCATCCCTCA	CCCTTCTCTGTCACCACCAT	2	44.9	Loc14_SNP1	TCTCTTTTACCTGGATATTCCAGAA(C/G)TGACTACTATGCAAGCTATAGCGTT	+	
Ptch1Loc15	ATCAGTCGAGCTGCTGTACG	TGCAGCCTCATAGTGGAATG	4	22.4	Loc15_SNP1	TCCTAAAAGTCCTATAATTACGCTA(A/T)ATCATAATGAACAGCATTTATTTAC	+	+
					Loc15_SNP2	TAAAATTAATTTCACTGATCAACTC(G/T)GTTTCAAATCAGCACAAACTGCCCA	+	+
Ptch1Loc16	CGATTTTGCTTGGCGTAGTC	TCCCATTTCCCATGTACACC	4	6.6	Loc16_SNP1	AGATCTTAAGTGGAATAGTCTGCAG(A/C)AACCAGCCCTGTACACACATGCACA	+	
					Loc16_SNP2	TGCTTCCATTTGTTTTGTCTCCAGC(A/G)CTGACGTACTGCAGTGAATATTTTA	+	
					Loc16_SNP3	AAGTTAGTTTAAAGAAAATAATTGC(C/T)TTCGTTTCATAAAACTTGAGATGAT	+	

*GenBank accession nos.: JN037665-JN037688.

†Total number of polymorphic sites identified in amplimer; analyzed for Fig. S3 *B–D*.

‡Location (kb) on genomic scaffold from Tilapia (*Oreochromis niloticus*) draft genome assembly.

§Polymorphic sites with a minor allele frequency >0.05 in *Metriaclima* and *Labeotropheus* populations; analyzed for Fig. 1*E*, main text.

¶Polymorphic sites with a minor allele frequency >0.05 in *Tropheops* populations; analyzed for Fig. 1*F*, main text.

‖Ptch1Loc6_SNP1 is also the SNP AIn100281_1741 discussed in main text.

**Ptch1Loc10_SNP1 was used as the "*Ptch1*" marker for quantitative trait loci analysis (Fig. 1*D*, main text) and is the high association SNP circled in Fig. 1 *E* and *F* (main text) and genotyped for Fig. 2 (main text).

Table S2. Species and populations used in study

Species	Location	Niche	*n*	Trophic mode	Comparison
Astatotilapia calliptera	Riverine (Malawi)	—	1	—	Fig. S1
Aulonocara hansbaenschi	Otter Point	Rock/Sand	1	Insectivore	Fig. S1
Benthochromis tricoti	Lake Tanganyika	—	1	—	Fig. S1
Cynotilapia afra	Thumbi West	Rock	2	Suction feeding	Fig. 2
Cynotilapia afra	Chiofu Bay	Rock	1	Suction feeding	Fig. 2
Cynotilapia afra	Chinyamwezi	Rock	22	Suction feeding	Fig. 2
Fossorochromis rostratus	Chembe	Sand	1	Sifter	Fig. S1
Hemitilapia oxyrhynchus	Masasa	Sand	1	Leaf cleaner	Fig. S1
Labeotropheus fuelleborni	Makanjila Point	Rock	6	Biting	Fig. 1*E*
Labeotropheus fuelleborni	Nankoma Rib.G	Rock	6	Biting	Fig. 1*E*
Labeotropheus fuelleborni	Makanjila Point	Rock	13	Biting	Fig. 2
Labeotropheus fuelleborni	Chiofu Bay	Rock	13	Biting	Fig. 2
Labeotropheus fuelleborni	Eccles Reef	Rock	5	Biting	Fig. 2
Labeotropheus fuelleborni	Chinyamwezi	Rock	14	Biting	Fig. 2
Labeotropheus fuelleborni	Chinyankwazi	Rock	11	Biting	Fig. 2
Labeotropheus trewavasae	Thumbi West	Rock	6	Biting	Fig. 1*E*
Labeotropheus trewavasae	Nakantenga	Rock	6	Biting	Fig. 1*E*
Lethrinops auritus	Otter Island	Sand	1	Sifter	Fig. S1
Melanochromis auratus	Otter Point	Rock	1	Omnivore	Fig. S1
Metriaclima aurora	Thumbi West	Rock	1	Suction feeding generalist	Fig. 2
Metriaclima aurora	Otter Point	Rock	7	Suction feeding generalist	Fig. 2
Metriaclima benetos	Mazinzi Reef	Rock	6	Suction feeding generalist	Fig. 1*E*
Metriaclima benetos	Mazinzi Reef	Rock	9	Suction feeding generalist	Fig. 2
Metriaclima mbenjii	Mbenji Island	Rock	6	Suction feeding generalist	Fig. 1*E*
Metriaclima pyrsonotus	Nakantenga	Rock	6	Suction feeding generalist	Fig. 1*E*
Metriaclima zebra	Thumbi West	Rock	6	Suction feeding generalist	Fig. 1*E*
Ophthalmotilapia ventralis	Lake Tanganyika	—	1	—	Fig. S1
Paracyprichromis nigrapinnis	Lake Tanganyika	—	1	—	Fig. S1
Petrochromis famula	Lake Tanganyika	—	1	—	Fig. S1
Petrotilapia tridentiger	Ikambe Sanga	Rock	1	Aufwuchs comber	Fig. S1
Protomelas taeniolatus	Thumbi West	Sand	1	Suction feeding	Fig. S1
Pseudotropheus williamsi	Thumbi West	Rock	1	Insectivore	Fig. S1
Rhamphochromis esox	Otter Point	Pelagic	1	Piscivore	Fig. S1
Serranochromis robustus	Riverine (Malawi)	—	1	—	Fig. S1
Tanganicodus irsacae	Lake Tanganyika	—	1	—	Fig. S1
Tropheops gracilior	Otter Island	Rock	12	Biting/suction	Fig. 1*F*, Fig. 2
Tropheops intermedius	Thumbi West	Rock	12	Biting/suction	Fig. 1*F*, Fig. 2
Tropheops sp.	Chinyankwazi	Rock	15	Biting/suction	Fig. 1*F*, Fig. 2
Tropheops sp.	Chinyamwezi	Rock	20	Biting/suction	Fig. 1*F*, Fig. 2

Table S3. Pixel density analysis of WISH

Species	Treatment*	Gene	Tissue†	*n*	Proportion labeled pixels (mean ± SE)	*t*	DF	*P* (*t* test)‡
Metriaclima zebra	None	*Ptch1*	Lower jaw	7	0.260 ± 0.032	**4.285**	**13**	**0.0009**
Labeotropheus fuelleborni	None	*Ptch1*	Lower jaw	8	0.448 ± 0.030			
Metriaclima zebra	None	*Ptch1*	Tail	7	0.134 ± 0.006	0.906	13	0.39
Labeotropheus fuelleborni	None	*Ptch1*	Tail	8	0.142 ± 0.006			
Metriaclima zebra	None	*Gli1*	Lower jaw	5	0.216 ± 0.020	**4.604**	**8**	**0.002**
Labeotropheus fuelleborni	None	*Gli1*	Lower jaw	5	0.357 ± 0.023			
Metriaclima zebra	None	*Gli1*	Tail	5	0.115 ± 0.010	0.335	8	0.74
Labeotropheus fuelleborni	None	*Gli1*	Tail	5	0.119 ± 0.005			
Metriaclima zebra	None	*Col1a1*	RA	3	0.212 ± 0.012	**3.434**	**6**	**0.027**
Labeotropheus fuelleborni	None	*Col1a1*	RA	3	0.283 ± 0.017			
Metriaclima zebra	None	*Col1a1*	Tail	3	0.327 ± 0.005	**3.877**	**6**	**0.018**
Labeotropheus fuelleborni	None	*Col1a1*	Tail	3	0.286 ± 0.010			
Labeotropheus fuelleborni	Cyc	*Ptch1*	Lower jaw	5	0.122 ± 0.019	**5.926**	**8**	**0.0004**
Labeotropheus fuelleborni	Control	*Ptch1*	Lower jaw	5	0.417 ± 0.048			
Labeotropheus fuelleborni	Cyc	*Ptch1*	Tail	5	0.027 ± 0.005	**15.844**	**8**	**0.0001**
Labeotropheus fuelleborni	Control	*Ptch1*	Tail	5	0.118 ± 0.009			
Labeotropheus fuelleborni	Cyc	*Gli1*	Lower jaw	5	0.208 ± 0.022	**5.011**	**8**	**0.001**
Labeotropheus fuelleborni	Control	*Gli1*	Lower jaw	5	0.373 ± 0.024			
Labeotropheus fuelleborni	Cyc	*Gli1*	Tail	5	0.033 ± 0.002	**13.815**	**8**	**< 0.0001**
Labeotropheus fuelleborni	Control	*Gli1*	Tail	5	0.127 ± 0.006			
Labeotropheus fuelleborni	Cyc	*Col1a1*	RA	3	0.162 ± 0.012	**5.325**	**6**	**0.006**
Labeotropheus fuelleborni	Control	*Col1a1*	RA	3	0.294 ± 0.021			
Labeotropheus fuelleborni	Cyc	*Col1a1*	Tail	3	0.339 ± 0.009	**4.062**	**6**	**0.015**
Labeotropheus fuelleborni	Control	*Col1a1*	Tail	3	0.289 ± 0.009			

*See *Materials and Methods* in main text (Cyc, 50 μM cyclopamine in ethanol; Control, ethanol control).
†See *SI Materials and Methods* for exact regions analyzed.
‡Boldface indicates significance based on a threshold of $P < 0.05$.

Table S4. Quantitative PCR gene expression analysis in microdissected tissue

Species	Gene	Tissue	*n*	Expression (mean ± SE)*	*t*	DF	*P* (*t* test)†
Metriaclima zebra	*Ptch1*	Lower jaw	6	1.033 ± 0.050	**−3.177**	**9**	**0.011**
Labeotropheus trewavasae	*Ptch1*	Lower jaw	5	1.268 ± 0.055			
Metriaclima zebra	*Ptch1*	Trunk/tail	6	1.023 ± 0.085	0.729	9	0.48
Labeotropheus trewavasae	*Ptch1*	Trunk/tail	5	0.931 ± 0.093			
Metriaclima zebra	*Rad26l*	Lower jaw	6	1.015 ± 0.036	−0.944	9	0.37
Labeotropheus trewavasae	*Rad26l*	Lower jaw	5	1.066 ± 0.040			
Metriaclima zebra	*Rad26l*	Trunk/tail	6	1.036 ± 0.076	−1.095	9	0.30
Labeotropheus trewavasae	*Rad26l*	Trunk/tail	5	1.160 ± 0.084			

*Relative transcript level vs. *M. zebra* average for each gene and tissue, normalized to β-actin transcript level as endogenous control (*SI Materials and Methods*).
†Boldface indicates significance based on a threshold of $P < 0.05$.

Table S5. Lower jaw measurements in larval fish

Species	Treatment*	*n*	Outlever (μm) (mean ± SD)	Inlever (μm) (mean ± SD)	MA_O (mean ± SD)
Labeotropheus fuelleborni	untreated control	6	1147.9 ± 83.0	288.2 ± 22.0	0.251 ± 0.019
Labeotropheus fuelleborni	ethanol control	7	1176.9 ± 339.4	296.5 ± 94.2	0.250 ± 0.035
Labeotropheus fuelleborni	50 μM cyclopamine	10	1127.2 ± 131.1	212.3 ± 24.4	0.190 ± 0.023
Metriaclima zebra	untreated control	7	1516.1 ± 70.2	311.6 ± 33.6	0.206 ± 0.026

MA_O, mechanical advantage of opening.
*See *Materials and Methods* in main text.