Supporting Information

Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika cichlid fishes

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1 Supplementary Text

Supplementary Text 1: Phylogenetic hypotheses for Lake Tanganyika cichlid fishes.
Nishida (1991) was the first to study tribal relationships in Tanganyikan cichlids based on genetic information, in this case allozyme data. In the resultant tree topology (Supplementary Figure S1A), the only included species of the tribe Tylochromini (*Tylochromis polylepis*) — a likely secondary colonizer of Lake Tanganyika (Koch et al. 2007) — was placed as the most ancestral taxon, followed by the only member of Boulengerochromini (*Boulengerochromis microlepis*), the Bathybatini, a clade comprised by Trematocarini and Tilapiini (represented by *Oreochromis tanganicae*, another secondary colonizer), Lamprologini, and a clade that combines the remaining tribes, referred to as 'H-lineage' (Nishida 1991). Within this H-lineage, exclusively consisting of moutbrooding species, Nishida (1991) proposed the following relationships: Limnochromini, together with Ectodini, formed the basal branch, followed by Cyprichromini, Perissodini and a clade in which Eretmodini is placed as sister group to Haplochromini. Several studies have used mitochondrial (mt) DNA markers to address the phylogenetic relationships between cichlid tribes in Lake Tanganyika (Sturmbauer and Meyer 1993; Kocher et al. 1995; Salzburger et al. 2002; Day et al. 2008) or a combination of mtDNA and one (Clabaut et al. 2005) or two (Muschick et al. 2012) nuclear markers (Supplementary Figure S1B-G). All these studies agree upon a basal position of the relatively species-poor tribes Bathybatini, Boulengerochromini, Trematocarini, as well as *O. tanganicae* and *T. polylepis* (note, however, that not all of these studies included representatives of all five taxa), yet their relative positions to one another differed. All but one (Sturmbauer and Meyer 1993) of these studies placed Eretmodini outside the H-lineage, either as sister group to Lamprologini (Kocher et al. 1995; Clabaut et al. 2005; Day et al. 2008) or as sister group to a clade combining Lamprologini and the remaining taxa of the H-lineage (Salzburger et al. 2002; Muschick et al. 2012). The relative order of taxa within the H-lineage varied between the different studies (see, e.g., Fig. 1 in Meyer et al. 2015).

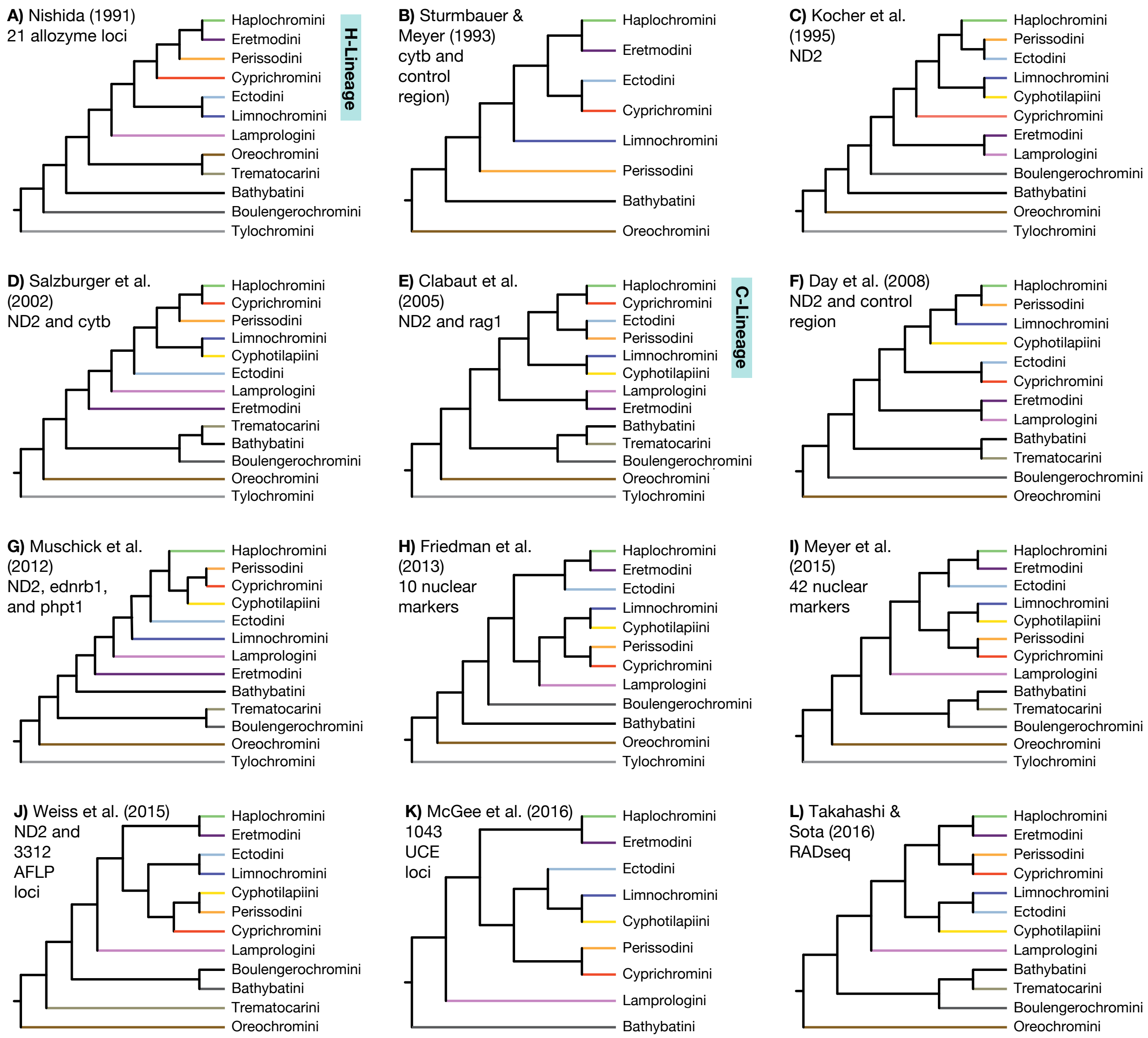
More recently, the problem of the tribal relationships within the cichlid assemblage of Lake Tanganyika has been addressed using larger numbers of nuclear DNA markers. Friedman et al. (2013) used 10 nuclear markers to resolve phylogenetic relationships in a much larger context, but had most Tanganyikan cichlid tribes represented in their data set. In their phylogeny, the genus *Tylochromis* appeared as the sister clade to all other cichlid lineages in Lake Tanganyika, followed by *O. tanganicae*, Bathybatini, Boulengerochromini, a clade formed by Lamprologini as sister group to the combined Cyprichromini, Perissodini, Cyphotilapiini, and Limnochromini, and a clade comprised by Ectodini as sistergroup to Eretmodini and Haplochromini (Supplementary Figure S1H). Meyer et al. (2015), using sequence information from 42 nuclear loci, presented a phylogenetic hypothesis, in which, again, *T. polylepis* and *O. tanganicae* branched off first, followed by a clade formed by Bathybatini and Trematocarini, while Boulengerochromini were placed as sister group to Lamprologini and the H-lineage (Supplementary Figure S1I). Therein, a clade comprising Perissodini and Cyprichromini was recovered as sister taxon to all other H-lineage tribes, Cyphotilapiini clustered with Limnochromini, and Eretmodini formed a clade with Haplochromini. However, when inspecting concordance between individual gene trees, certain discrepancies were found. For example, the placement of Eretmodini as sister group to Haplochromini was only supported in a subset of 14 congruent gene trees, whereas another 13 gene trees supported Eretmodini as most basal taxon within the H-lineage (Meyer et al. 2015). McGee et al. (2016), in a comparison of jaw morphology between cichlids from Lake Tanganyika and Malawi, used sequence information from a large number of ultraconserved elements. In their phylogeny containing sequence information from 56 Tanganyika and Malawi cichlids representing 10 different tribes (and using a representative of Bathybatini as outgroup), the Lamprologini were placed as sistergroup to a clade containing the H-lineage taxa, in which two sub-clades were recovered: A clade with Perissodini and Cyprichromini as sistergroup to Ectodini and Cyphotilapiini with Limnochromini, and a clade with Eretmodini and Haplochromini (McGee et al. 2016) (Supplementary Figure S1K).

In contrast to the above studies, Weiss et al. (2015) used AFLP markers to resolve phylogenetic relationships between the cichlid tribes of Lake Tanganyika, and to place them into a larger phylogenetic context. Their neighbor-joining consensus phylogeny (not including *T. polylepis* and using Oreochromini as outgroup) suggests that Trematocarini form an independent lineage, separated from the remaining tribes by riverine Austrotilapiines (Supplementary Figure S1J). A clade formed by Bathybatini and Boulengerochromini was placed as sister group to the Lamprologini and the H-lineage; followed by a clade comprised by Ectodini, Limnochromini, Cyprichromini, Benthochromini, Perissodini, and Cyphotilapiini; and the Eretmodini as sister group to the Haplochromini. The study of Weiss et al. (2015) is, thus, the first to suggest monophyly of what they call ‘ancient Tanganyika mouthbrooders’ (all members of the H-lineage except Eretmodini and Haplochromini). Takahashi and Sota (2016) used RAD sequencing data to infer the phylogenetic history of Lake Tanganyika cichlid tribes. With millions of concatenated base pairs and maximum likelihood inference, they produced a tree estimate with generally high bootstrap support (Supplementary Figure S1L). Their work provided further support for relationships identified in previous studies, including a monophyletic clade combing Bathybatini, Boulengerochromini and Trematocarini (Salzburger et al. 2002; Clabaut et al. 2005; Meyer et al. 2015) and a sister group relationship between Ectodini and Limnochromini (Weiss et al. 2015). However, in contrast to Weiss et al. (2015), Takahashi and Sota (2016) recovered Perissodini and Cyprichromini as more closely related to Haplochromini and Eretmodini than to other H-lineage taxa, and therefore did not find support for the monophyly of the so-called ‘ancient Lake Tanganyika mouthbrooders’.

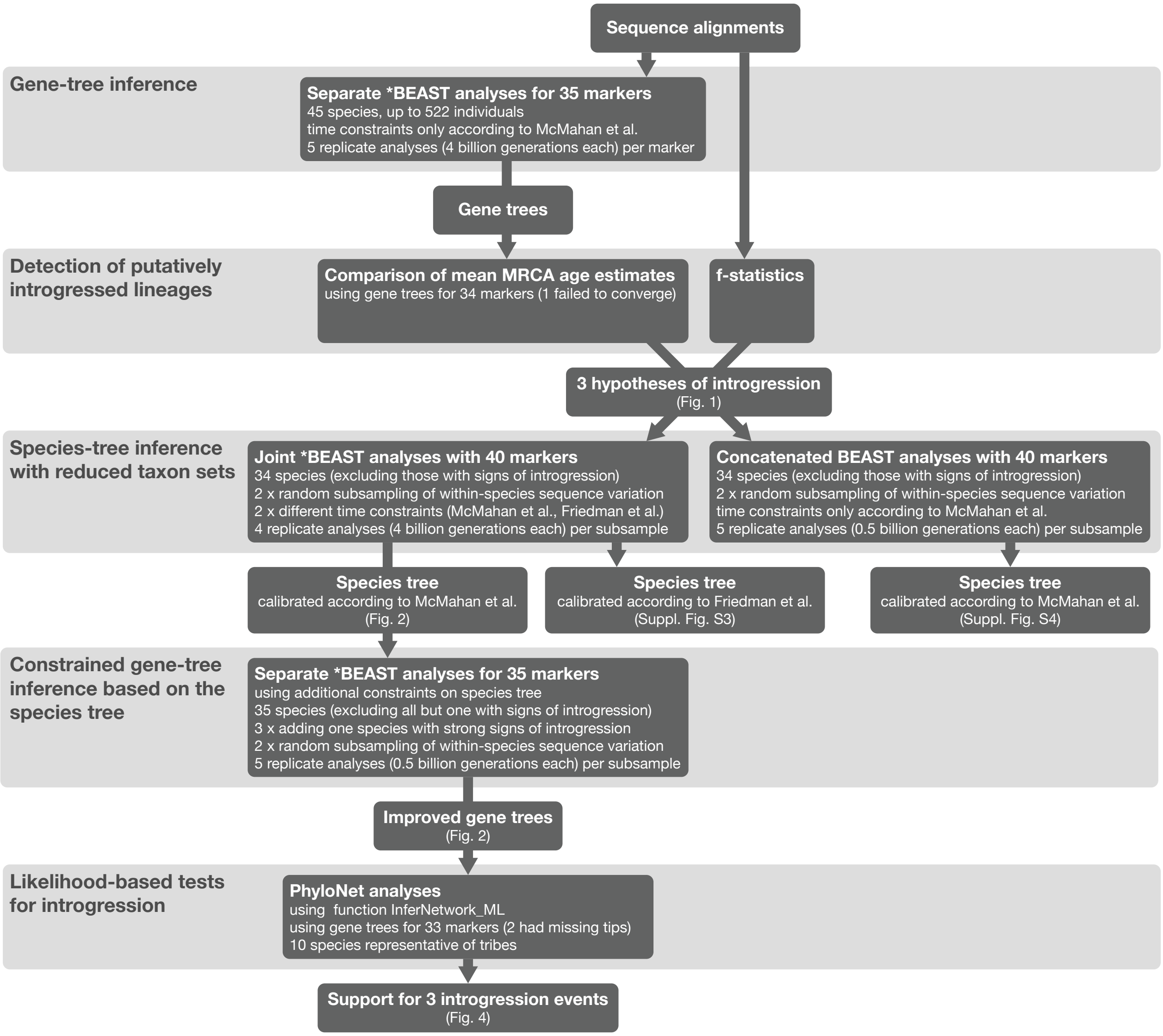
2 Supplementary Figures

Supplementary Figure S1: Previous hypotheses for the phylogenetic relationships among cichlid tribes in Lake Tanganyika.

The figure depicts simplified cladograms based on the studies of A) Nishida (1991), B) Sturmbauer and Meyer (1993), C) Kocher et al. (1995), D) Salzburger et al. (2002), E) Clabaut et al. (2005), F) Day et al. (2008), G) Muschick et al. (2012), H) Friedman et al. (2013), I) Meyer et al. (2015), J) Weiss et al. (2015), K) McGee et al. (2016), L) Takahashi and Sota (2016). The markers used in the respective study are indicated; the color code for cichlid tribes follows that of Muschick et al. (2012).

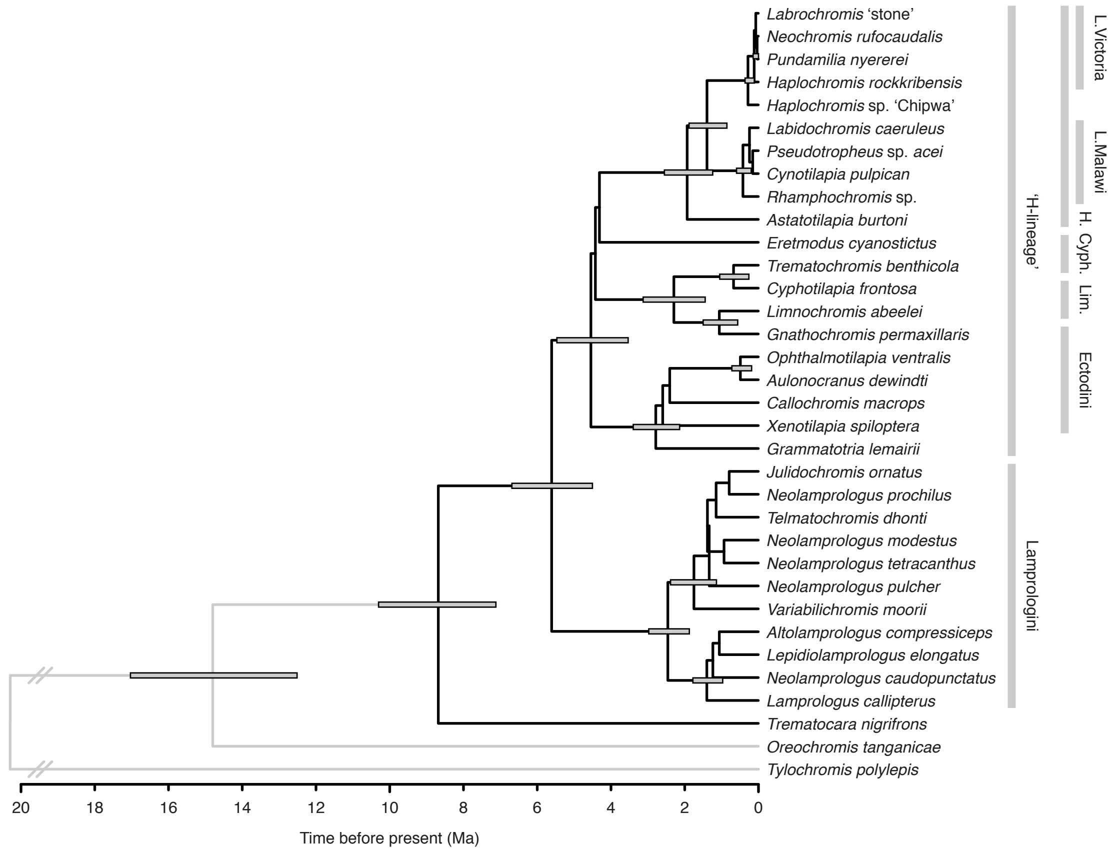


Supplementary Figure S2: Flow chart illustrating our phylogenetic analysis pipeline. Labels on the left match the corresponding section titles in the Materials and Methods and in the Results.



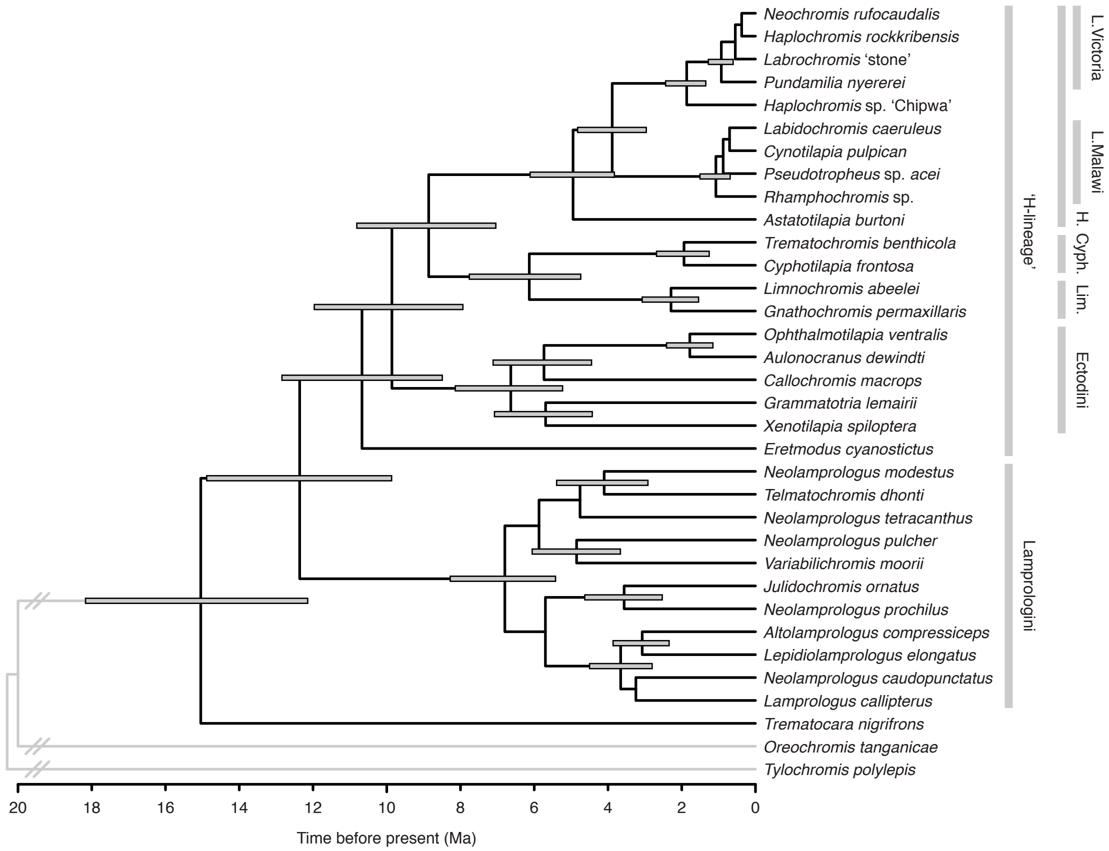
Supplementary Figure S3: Species tree of reduced taxon set inferred with time constraints according to Friedman et al. (2013).

Gray bars indicate 95% HPD intervals of divergence age estimates for all nodes supported with BPP ≥ 0.99. The divergence of *Tylochromis polylepis* is not drawn to scale. L., Lake; H., Haplochromini; Cyph., Cyphotilapiini; Lim., Limnochromini.

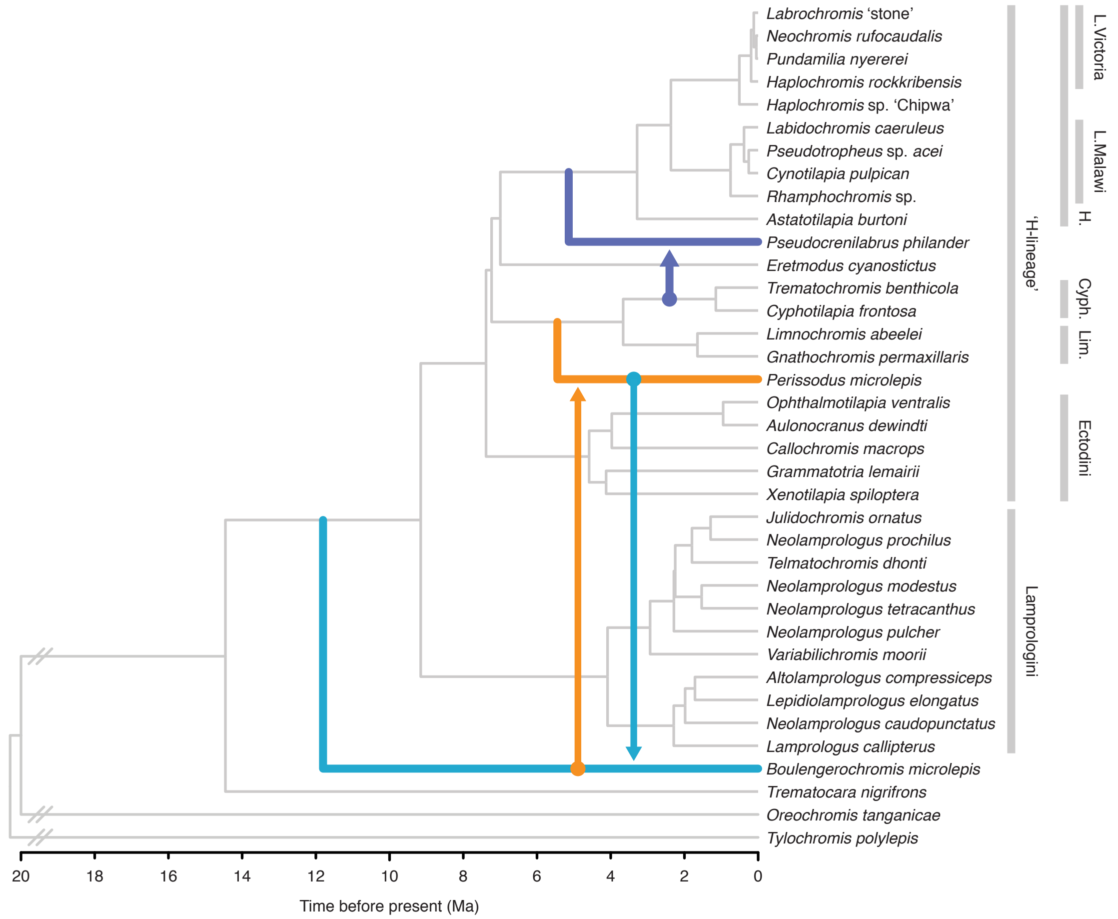


Supplementary Figure S4: Species tree of reduced taxon set inferred with concatenated sequence data.

Gray bars indicate 95% HPD intervals of divergence age estimates for all nodes supported with BPP ≥ 0.99. The divergences of *Oreochromis tanganicae* and *Tylochromis polylepis* are not drawn to scale. L., Lake; H., Haplochromini; Cyph., Cyphotilapiini; Lim., Limnochromini.



Supplementary Figure S5: Species tree inferred with the multi-species coalescent model, illustrating the three inferred introgression events.
The species tree is identical to the tree shown in Fig. 2. The positions of the three species affected by introgression *B. microlepis*, *P. microlepis*, and *P. philander* are indicated according to our likelihood-based tests for introgression (Fig. 4), and under the assumption that introgression occurred reciprocally between *B. microlepis* and *P. microlepis* (see Discussion). The timing of the attachment points is chosen arbitrarily, as it was not inferred in our likelihood-based tests for introgression. Divergence of *Oreochromis tanganicae* and *Tylochromis polylepis* are not drawn to scale. L., Lake; H., Haplochromini; Cyph., Cyphotilapiini; Lim., Limnochromini.



3 Supplementary Tables

Supplementary Table S1 (next page): Number and origin of individuals sampled for each of 45 cichlid species.

The number of sequenced individuals is given as *n*, with the number of aquaria-bred individuals in parentheses. Abbreviations for places of origin are explained in Supplementary Table S2. Places of origin "EAWAG" and "Basel" refer to individuals bred in aquaria at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG), or at the aquarium facilities of the University of Basel. LT, Lake Tanganyika; LM, Lake Malawi; LV, Lake Victoria.

Supplementary Table S1: Number and origin of individuals sampled for each of 45 cichlid species.

Tribe	Species	*n*	Year	Place of origin	Lake
Tylochromini	*Tylochromis polylepis*	7	2007/08	T,M	LT
Tilapiini	*Oreochromis tanganicae*	8	2008	M	LT
Trematocarini	*Trematocara nigrifrons*	16	2008	M	LT
Boulengerochromini	*Boulengerochromis microlepis*	8	2007/08	M,T	LT
Bathybatini	*Bathybates graueri*	16	2007/08	M	LT
Lamprologini	*Lamprologus callipterus*	15	2007/08	MIW,T,KTL,W	LT
Lamprologini	*Neolamprologus caudopunctatus*	6	2007	LB	LT
Lamprologini	*Lepidiolamprologus elongatus*	16	2007/08	MIN,LB,KTL,MIW,T,M,	LT
Lamprologini	*Altolamprologus compressiceps*	16	2007/08	M,KTL,MIN,LB,MIW,T	LT
Lamprologini	*Julidochromis ornatus*	16 (1)	2007/08	MIW,T,MIE,KTL,Basel	LT
Lamprologini	*Neolamprologus prochilus*	7	2008/11	M,T	LT
Lamprologini	*Variabilichromis moorii*	15	2007/08	KTL,MIN,MIW,T	LT
Lamprologini	*Neolamprologus pulcher*	16 (6)	2008	T,Basel	LT
Lamprologini	*Telmatochromis dhonti*	16	2007/08	MIN,KTL,M,MIW,T	LT
Lamprologini	*Neolamprologus modestus*	8	2007/08	KTL,MIW	LT
Lamprologini	*Neolamprologus tetracanthus*	8	2007/08	MIW,LB,KTL,T	LT
Cyprichromini	*Cyprichromis leptosoma*	10	2008	T	LT
Perissodini	*Haplotaxodon microlepis*	16	2007/08	M,T	LT
Perissodini	*Perissodus microlepis*	16	2007/08	MIN,LB,KTL,MIW,T,K	LT
Limnochromini	*Gnathochromis permaxillaris*	16	2008	M	LT
Limnochromini	*Limnochromis abeelei*	8	2007	M	LT
Cyphotilapiini	*Cyphotilapia frontosa*	16	2007/08	M,T	LT
Cyphotilapiini	*Trematochromis benthicola*	8	2008/11	M	LT
Ectodini	*Xenotilapia spiloptera*	16	2007/08	T,M,K	LT
Ectodini	*Grammatotria lemairii*	16	2007/08	M	LT
Ectodini	*Callochromis macrops*	16 (1)	2007/08	T,KTL,MIN,LB,Basel	LT
Ectodini	*Aulonocranus dewindti*	16	2007/08	MIN,LB,MIW,T,K,MIE	LT
Ectodini	*Ophthalmotilapia ventralis*	16	2007/08	KTL,T,MIW,W,MIN	LT
Eretmodini	*Eretmodus cyanostictus*	16	2007/08	KTL,MIN,MIW,T	LT
Haplochromini	*Pseudocrenilabrus philander*	14	2007	KR	-
Haplochromini	*Serranochromis macrocephalus*	16	2007	KR	-
Haplochromini	*Tropheus moorii*	16	2007/08	KTL,MIN,MIW,T	LT
Haplochromini	*Lobochilotes labiatus*	16	2007/08	KTL,M,MIN,MIW,T,MIE	LT
Haplochromini	*Gnathochromis pfefferi*	16	2008	T,MIW,K	LT
Haplochromini	*Ctenochromis horei*	16	2007/08	KTL,MIN,LB,MIW,T	LT
Haplochromini	*Astatotilapia burtoni*	16	2007	C	LT
Haplochromini	*Labidochromis caeruleus*	2 (2)		Basel	LM
Haplochromini	*Rhamphochromis* sp.	1 (1)		Basel	LM
Haplochromini	*Pseudotropheus* sp. *acei*	3 (3)		Basel	LM
Haplochromini	*Cynotilapia pulpican*	2 (2)		Basel	LM
Haplochromini	*Haplochromis* sp. ‘Chipwa’	8	2011/12	C,LD	LT
Haplochromini	*Neochromis rufocaudalis*	4 (4)		EAWAG	LV
Haplochromini	*Haplochromis rockkribensis*	4 (4)		EAWAG	LV
Haplochromini	*Labrochromis* ‘stone’	4 (4)		EAWAG	LV
Haplochromini	*Pundamilia nyererei*	4 (4)		EAWAG,Basel	LV
Total		522 (32)			

Supplementary Table S2: Places of origin for sequenced cichlid individuals.
Abbreviations listed here are used in Supplementary Table S1. With the exception of Lufubu delta and Kafue River, all sampling locations are within Lake Tanganyika.

Abbreviation	Name of location	Coordinates
M	Mpulungu area	S 8.75699 E 31.11389
KTL	Kasakalawe Tanganyika Lodge	S 8.78107 E 31.09151
MIE	Mbita Island NE	S 8.74700 E 31.10123
MIW	Mbita Island W	S 8.75335 E 31.08622
MIN	Mbita Island N	S 8.74966 E 31.08768
C	Chipwa	S 8.60174 E 31.18701
T	Toby's Lodge	S 8.62323 E 31.20045
W	Woynze	S 8.72519 E 31.13338
K	Kasenga	S 8.71525 E 31.14187
LB	Luke's Beach	S 8.60896 E 31.19594
LD	Lufubu delta	S 8.56226 E 30.72178
KR	Kafue River	S 15.7805 E 28.15366

Supplementary Table S3 (next page): Differences in mean MRCA age estimates in three-taxon comparisons.
For each species, the maximum difference Δt (in myr) in mean MRCA age estimates in three-taxon comparisons is given, together with the two other taxa involved in the comparison, and the mean MRCA age estimates (in Ma) between species A and B (A,B), A and C (A,C), and B and C (B,C).

Supplementary Table S3: Differences in mean MRCA age estimates in three-taxon comparisons.

Taxon A	Δt	Taxon B	Taxon C	(A,B)	(A,C)	(B,C)
Oreochromis tanganicae	0.60	*Rhamphochromis* sp.	*T. polylepis*	27.17	41.90	42.50
Trematocara nigrifrons	0.40	*Rhamphochromis* sp.	*T. polylepis*	18.20	42.10	42.50
Boulengerochromis microlepis	2.13	*T. nigrifrons*	*P. microlepis*	15.10	15.54	17.67
Bathybates graueri	1.46	*T. nigrifrons*	*H. microlepis*	13.95	16.18	17.67
Lamprologus callipterus	0.58	*Rhamphochromis* sp.	*T. polylepis*	16.98	41.92	42.50
Neolamprologus caudopunctatus	0.52	*J. ornatus*	*X. spiloptera*	11.42	16.13	16.65
Lepidiolamprologus elongatus	0.89	*N. caudopunctatus*	*N. prochilus*	7.45	10.21	11.10
Altolamprologus compressiceps	1.24	*N. caudopunctatus*	*N. prochilus*	7.85	9.86	11.10
Julidochromis ornatus	0.94	*N. prochilus*	*N. modestus*	7.34	8.92	9.86
Neolamprologus prochilus	1.26	*N. modestus*	*A. compressiceps*	9.86	9.86	11.12
Variabilichromis moorii	0.90	*J. ornatus*	*G. permaxillaris*	9.18	15.76	16.66
Neolamprologus pulcher	1.15	*N. prochilus*	*N. modestus*	8.55	8.70	9.86
Telmatochromis dhonti	1.11	*N. modestus*	*N. prochilus*	8.31	8.75	9.86
Neolamprologus modestus	0.79	*N. caudopunctatus*	*G. lemairii*	11.47	15.92	16.71
Neolamprologus tetracanthus	1.08	*N. caudopunctatus*	*G. lemairii*	11.13	15.64	16.71
Cyprichromis leptosoma	1.81	*P. nyererei*	*B. graueri*	15.84	16.32	18.13
Haplotaxodon microlepis	1.95	*P. nyererei*	*B. graueri*	16.12	16.18	18.13
Perissodus microlepis	1.99	*G. pfefferi*	*B. microlepis*	15.34	15.54	17.53
Gnathochromis permaxillaris	1.58	*H. microlepis*	*V. moorii*	14.29	15.76	17.35
Limnochromis abeelei	1.75	*N. rufocaudalis*	*B. microlepis*	15.82	16.10	17.84
Cyphotilapia frontosa	1.63	*L. abeelei*	*P. philander*	13.37	13.87	15.50
Trematochromis benthicola	1.73	*P. philander*	*L. abeelei*	13.76	13.77	15.50
Xenotilapia spiloptera	1.55	*N. caudopunctatus*	*C. leptosoma*	15.74	16.13	17.68
Grammatotria lemairii	1.74	*C. leptosoma*	*J. ornatus*	15.43	15.88	17.62
Callochromis macrops	1.24	*G. lemairii*	*A. dewindti*	11.00	11.02	12.26
Aulonocranus dewindti	1.16	*C. leptosoma*	*N. caudopunctatus*	15.65	16.53	17.68
Ophthalmotilapia ventralis	1.36	*C. leptosoma*	*N. caudopunctatus*	15.55	16.32	17.68
Eretmodus cyanostictus	1.11	*C. leptosoma*	*J. ornatus*	14.88	16.51	17.62
Pseudocrenilabrus philander	1.77	*N. rufocaudalis*	*T. benthicola*	13.71	13.76	15.53
Serranochromis macrocephalus	1.24	*N. rufocaudalis*	*C. frontosa*	13.50	14.36	15.60
Tropheus moorii	1.37	*N. rufocaudalis*	*C. frontosa*	13.57	14.23	15.60
Lobochilotes labiatus	1.68	*N. rufocaudalis*	*C. frontosa*	13.21	13.92	15.60
Gnathochromis pfefferi	1.23	*H. rockkribensis*	*E. cyanostictus*	13.21	14.38	15.61
Ctenochromis horei	1.18	*N. rufocaudalis*	*C. frontosa*	12.93	14.42	15.60
Astatotilapia burtoni	1.53	*T. moorii*	*H. rockkribensis*	12.11	12.12	13.65
Labidochromis caeruleus	0.74	*H. rockkribensis*	*A. burtoni*	10.66	11.39	12.12
Rhamphochromis sp.	1.09	*A. burtoni*	*H. rockkribensis*	10.73	11.04	12.12
Pseudotropheus sp. *acei*	1.12	*H. rockkribensis*	*A. burtoni*	10.63	11.00	12.12
Cynotilapia pulpican	0.87	*H. rockkribensis*	*A. burtoni*	10.47	11.25	12.12
Haplochromis sp. ‘Chipwa’	1.00	*P. nyererei*	*Rhamphochromis* sp.	6.11	10.10	11.10
Neochromis rufocaudalis	0.66	*C. leptosoma*	*L. callipterus*	15.82	16.95	17.61
Haplochromis rockkribensis	0.63	*C. leptosoma*	*L. callipterus*	15.85	16.99	17.61
Labrochromis ‘stone’	0.81	*C. leptosoma*	*L. callipterus*	15.63	16.80	17.61
Pundamilia nyererei	0.66	*C. leptosoma*	*L. callipterus*	15.84	16.96	17.61

Supplementary Table S4: f_4-statistics.

The f_4-statistic was calculated in four-taxon comparisons to test three hypotheses of introgression. We used 24 comparisons to test for hypothesis H1, and 16 comparisons for both of hypotheses H2 and H3 (see main text). For each comparison, the number of bi-allelic SNPs used for the test and the percentage of SNPs that are variable in both pairs of sister species (% var) are given. Significance of f_4 values was assessed with a block Jackknife procedure (p_{JK}) and with ILS-based simulations (p_{sim}). Normality of Jackknife block f_4-values was tested using the Shapiro-Wilks test (p_{norm}).

Hypothesis H1

Taxon A	Taxon B	Taxon C	Taxon D	SNPs	% var	f_4	p_{JK}	p_{norm}	p_{sim}
O. tanganicae	*B. graueri*	*L. abeelei*	*L. elongatus*	716	5.3	-0.002	0.432	0.000	0.448
O. tanganicae	*B. graueri*	*L. abeelei*	*N. prochilus*	676	3.4	0.010	0.919	0.000	0.207
O. tanganicae	*B. graueri*	*P. microlepis*	*L. elongatus*	745	4.4	-0.008	0.203	0.000	0.300
O. tanganicae	*B. graueri*	*P. microlepis*	*N. prochilus*	702	3.0	0.001	0.538	0.000	0.486
O. tanganicae	*B. microlepis*	*L. abeelei*	*L. elongatus*	614	4.2	-0.005	0.274	0.000	0.361
O. tanganicae	*B. microlepis*	*L. abeelei*	*N. prochilus*	580	2.8	0.005	0.752	0.000	0.325
O. tanganicae	*B. microlepis*	*P. microlepis*	*L. elongatus*	652	3.5	-0.010	0.145	0.000	0.217
O. tanganicae	*B. microlepis*	*P. microlepis*	*N. prochilus*	615	2.1	0.001	0.550	0.000	0.458
T. nigrifrons	*B. graueri*	*L. abeelei*	*L. elongatus*	658	4.6	-0.010	0.096	0.001	0.258
T. nigrifrons	*B. graueri*	*L. abeelei*	*N. prochilus*	620	3.5	-0.008	0.120	0.000	0.222
T. nigrifrons	*B. graueri*	*P. microlepis*	*L. elongatus*	691	4.3	-0.007	0.171	0.002	0.299
T. nigrifrons	*B. graueri*	*P. microlepis*	*N. prochilus*	650	3.7	-0.005	0.261	0.000	0.293
T. nigrifrons	*B. microlepis*	*L. abeelei*	*L. elongatus*	569	4.4	-0.013	0.045	0.000	0.172
T. nigrifrons	*B. microlepis*	*L. abeelei*	*N. prochilus*	537	3.5	-0.014	0.012	0.000	0.148
T. nigrifrons	*B. microlepis*	*P. microlepis*	*L. elongatus*	610	4.6	-0.014	0.062	0.000	0.166
T. nigrifrons	*B. microlepis*	*P. microlepis*	*N. prochilus*	575	3.8	-0.011	0.097	0.000	0.171

Hypothesis H2

Taxon A	Taxon B	Taxon C	Taxon D	SNPs	% var	f_4	p_{JK}	p_{norm}	p_{sim}
O. tanganicae	*B. graueri*	*H. microlepis*	*G. lemairii*	708	4.4	-0.011	0.068	0.000	0.216
O. tanganicae	*B. graueri*	*H. microlepis*	*O. ventralis*	708	4.9	-0.011	0.078	0.000	0.237
O. tanganicae	*B. graueri*	*P. microlepis*	*G. lemairii*	695	4.3	-0.011	0.073	0.000	0.213
O. tanganicae	*B. graueri*	*P. microlepis*	*O. ventralis*	697	5.2	-0.012	0.069	0.001	0.222
O. tanganicae	*B. microlepis*	*H. microlepis*	*G. lemairii*	611	4.4	-0.012	0.123	0.000	0.210
O. tanganicae	*B. microlepis*	*H. microlepis*	*O. ventralis*	627	3.5	-0.016	0.020	0.000	0.127
O. tanganicae	*B. microlepis*	*P. microlepis*	*G. lemairii*	604	4.3	-0.013	0.090	0.000	0.201
O. tanganicae	*B. microlepis*	*P. microlepis*	*O. ventralis*	620	3.7	-0.018	0.017	0.000	0.107
T. nigrifrons	*B. graueri*	*H. microlepis*	*G. lemairii*	665	2.9	-0.001	0.337	0.000	0.446
T. nigrifrons	*B. graueri*	*H. microlepis*	*O. ventralis*	670	4.3	-0.003	0.272	0.000	0.389
T. nigrifrons	*B. graueri*	*P. microlepis*	*G. lemairii*	658	2.9	0.000	0.514	0.000	0.474
T. nigrifrons	*B. graueri*	*P. microlepis*	*O. ventralis*	663	4.1	-0.002	0.368	0.000	0.428
T. nigrifrons	*B. microlepis*	*H. microlepis*	*G. lemairii*	590	3.6	-0.002	0.320	0.000	0.419
T. nigrifrons	*B. microlepis*	*H. microlepis*	*O. ventralis*	601	4.5	-0.009	0.118	0.000	0.265
T. nigrifrons	*B. microlepis*	*P. microlepis*	*G. lemairii*	585	3.9	-0.005	0.176	0.000	0.313
T. nigrifrons	*B. microlepis*	*P. microlepis*	*O. ventralis*	595	4.7	-0.012	0.052	0.000	0.210

Supplementary Table S4: f_4-statistics (continued).

Hypothesis H3									
Taxon A	Taxon B	Taxon C	Taxon D	SNPs	% var	f_4	p_{JK}	p_{norm}	p_{sim}
G. lemairii	*C. frontosa*	*L. labiatus*	*H.* sp. 'Chipwa'	393	2.3	-0.001	0.419	0.000	0.485
G. lemairii	*C. frontosa*	*L. labiatus*	*A. burtoni*	401	3.5	-0.015	0.086	0.000	0.107
G. lemairii	*C. frontosa*	*P. philander*	*H.* sp. 'Chipwa'	491	2.6	-0.003	0.314	0.000	0.350
G. lemairii	*C. frontosa*	*P. philander*	*A. burtoni*	499	4.0	-0.008	0.147	0.000	0.232
G. lemairii	*T. benthicola*	*L. labiatus*	*H.* sp. 'Chipwa'	390	2.8	-0.007	0.108	0.000	0.254
G. lemairii	*T. benthicola*	*L. labiatus*	*A. burtoni*	398	4.3	-0.017	0.077	0.000	0.109
G. lemairii	*T. benthicola*	*P. philander*	*H.* sp. 'Chipwa'	491	2.0	0.002	0.676	0.000	0.394
G. lemairii	*T. benthicola*	*P. philander*	*A. burtoni*	499	4.2	-0.008	0.116	0.001	0.278
O. ventralis	*C. frontosa*	*L. labiatus*	*H.* sp. 'Chipwa'	403	3.7	0.000	0.505	0.002	0.471
O. ventralis	*C. frontosa*	*L. labiatus*	*A. burtoni*	403	4.2	-0.010	0.182	0.002	0.218
O. ventralis	*C. frontosa*	*P. philander*	*H.* sp. 'Chipwa'	499	3.8	-0.009	0.113	0.002	0.263
O. ventralis	*C. frontosa*	*P. philander*	*A. burtoni*	501	5.0	-0.017	0.018	0.003	0.143
O. ventralis	*T. benthicola*	*L. labiatus*	*H.* sp. 'Chipwa'	397	3.0	-0.006	0.259	0.003	0.291
O. ventralis	*T. benthicola*	*L. labiatus*	*A. burtoni*	396	5.1	-0.015	0.160	0.018	0.182
O. ventralis	*T. benthicola*	*P. philander*	*H.* sp. 'Chipwa'	495	2.8	-0.004	0.270	0.000	0.339
O. ventralis	*T. benthicola*	*P. philander*	*A. burtoni*	497	5.4	-0.019	0.019	0.020	0.135

4 Supplementary Files

Supplementary File S1: Compressed archive of alignments in Nexus format.
This archive contains the following three subdirectories:

- Alignments used for unconstrained gene-tree analyses (unconstrained_gene_tree_inference)
- Alignments used for species-tree analyses (species_tree_inference)
- Alignments used for constrained gene tree inference (constrained_gene_tree_inference)

See file **nexus.zip**

Supplementary File S2: Compressed archive of species-level MCC trees.
This archive contains seven files. Note that all trees are based on time constraints according to the timeline of McMahan et al. (2013) except where noted.

- Species-level MCC trees for unconstrained gene-tree analyses (unconstrained_gene_tree_inference_MCC.trees)
- MCC tree for species-tree analyses with reduced taxon set and the multi-species coalescent model (species_tree_inference_coalescent_MCC.tre)
- MCC tree for species-tree analyses with reduced taxon set and the multi-species coalescent model, based on time constraints according to Friedman et al. (2013) (species_tree_inference_coalescent_friedman_et_al_constraints_MCC.tre)
- MCC tree for species-tree analyses with reduced taxon set and concatenation (species_tree_inference_concatenation_MCC.tre)
- MCC trees for constrained gene-tree analyses with *Boulengerochromis microlepis* (constrained_gene_tree_inference_w_boulengerochromis_MCC.trees)
- MCC trees for constrained gene-tree analyses with *Perissodus microlepis* (constrained_gene_tree_inference_w_perissodus_MCC.trees)
- MCC trees for constrained gene-tree analyses with *Pseudocrenilabrus philander* (constrained_gene_tree_inference_w_pseudocrenilabrus_MCC.trees)

See file **trees.zip**

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