Molecular phylogeny of *Oreochromis* (Cichlidae: Oreochromini) reveals mito-nuclear discordance and multiple colonisation of adverse aquatic environments

Antonia G. P. Ford, Thomas R. Bullen, Longson Pang, Martin J. Genner, Roger Bills, Tomáš Flouri, Benjamin P. Ngatunga, Lukas Rüber, Ulrich K. Schliewen, Ole Seehausen, Asilatu Shechonge, Melanie L. J. Stiassny, George F. Turner, Julia J. Day

Appendix A Supplementary Information

Table of contents

Table S1. Specimen information and Genbank accession numbers.

	Specimen information					Genbank accession numbers							
Species	**Voucher/ Field *N***	**Locality**	**Country**	**GPS**		**mtDNA**		**nuDNA**					
				Latit.	**Longit.**	**ND2**	**16S**	**BMP4**	**CCNG1**	**GAPDHS**	**TYR**	**S7**	**b2m**
A. alcalica	AF 160	Lake Natron	Tanzania	-2.60	35.92	✓	✓	✓	✓	✓	✓	✓	-
A. alcalica	AF 881	Ewaso Ngiro River	Tanzania	-2.15	36.06	✓	✓	✓	✓	✓	✓	✓	✓
A. grahami	AF 856	Magadi Hot Springs	Kenya	-2.00	36.23	✓	✓	✓	✓	✓	✓	✓	x
A. grahami	AF 869	Magadi Hot Springs	Kenya	-2.00	36.23	✓	✓	✓	✓	x	✓	✓	✓
A. latilabris	AF 088	Lake Natron	Tanzania	-2.60	35.92	✓	✓	✓	✓	✓	✓	x	✓
A. latilabris	AF 122	Lake Natron	Tanzania	-2.60	35.92	✓	✓	✓	✓	✓	✓	✓	✓
A. ndalalani	AF 123	Lake Natron	Tanzania	-2.60	35.92	✓	✓	✓	✓	✓	✓	✓	✓
A. ndalalani	AF 124	Lake Natron	Tanzania	-2.60	35.92	✓	✓	✓	✓	✓	✓	✓	✓
O. amphimelas	AF 001	Lake Manyara	Tanzania	-3.42	35.85	✓	✓	✓	✓	✓	✓	✓	✓
O. amphimelas	AF 002	Lake Manyara	Tanzania	-3.42	35.85	✓	✓	✓	✓	✓	✓	x	x
O. amphimelas	AF 003	Lake Manyara	Tanzania	-3.42	35.85	✓	✓	✓	✓	✓	✓	✓	x
O. andersonii	SAIAB 133	Cunene Lagoon	Namibia	-17.26	11.76	✓	✓	✓	x	✓	✓	✓	✓
O. andersonii	SAIAB C5	Panhandle, Ngamiland,	Botswana	-18.43	21.90	✓	✓	✓	x	✓	✓	✓	✓
O. angolensis	SAIAB F203	Kawa River	Angola	-9.17	13.37	✓	✓	✓	✓	✓	✓	✓	✓
O. angolensis	SAIAB F204	Kwanza River	Angola	-9.17	13.37	✓	✓	✓	✓	✓	✓	✓	✓
O. aureus						AB195550							
O. aureus						AB195551							
O. aureus						AB195552							
O. aureus						DQ465029							
O. chungruruensis	MG 3D2A	Lake Kyungululu	Tanzania	9.18	33.51	✓	✓	✓	✓	✓	✓	✓	✓
O. chungruruensis	MG 3D2B	Lake Kyungululu	Tanzania	9.18	33.51	✓	✓	✓	✓	✓	-	✓	-
O. chungruruensis	ZSM 934	Lake Chungruru,	Tanzania	9.31	33.86	✓	X	✓	✓	✓	✓	✓	-
O. esculentus	MG 21A	Lake Rukwa	Tanzania	8.24	32.55	✓	✓	✓	✓	✓	✓	✓	✓

O. esculentus	MG 21B	Lake Rukwa	Tanzania	8.24	32.55	✓	✓	✓	✓	✓	✓	✓	✓
O. hunteri	MG 111333	Lake Chala	Tanzania	3.18	37.41	✓	✓	✓	✓	✓	✓	✓	✓
O. hunteri	MG 111334	Lake Chala	Tanzania	3.18	37.41	✓	✓	✓	✓	✓	✓	✓	x
O. hunteri	MG 111373	Lake Chala	Tanzania	3.18	37.41	✓	✓	✓	✓	✓	✓	✓	✓
O. cf. *jipe*	ZSM 1065	Lake Chala	Tanzania	-3.32,	37.70	✓	✓	-	-	-	-	-	-
O. jipe “pangani”	MG P2F2	Lake Kalimau	Tanzania	4.42	38.09	✓	✓	✓	✓	✓	✓	✓	✓
O. jipe “pangani”	MG P2F3	Lake Kalimau	Tanzania	4.42	38.09	✓	✓	✓	✓	✓	✓	✓	✓
O. jipe ”pangani”	MG P2G2	Nyumba-ya-Mungu	Tanzania	3.61	37.46	✓	✓	✓	✓	✓	✓	✓	✓
O. karomo	MG206	Lake Nyamagoma	Tanzania	4°59.903'	21°11.734'	✓	✓	✓	✓	✓	✓	✓	✓
O. karomo	MG207	Lake Nyamagoma	Tanzania	4°59.903'	21°11.734'	✓	✓	x	✓	✓	✓	✓	✓
O. karomo	MG208	Lake Nyamagoma	Tanzania	4°59.903'	21°11.734'	✓	✓	✓	✓	✓	✓	✓	✓
O. karongae	MG 226	Salima, Lake Malawi	Malawi	13°47'25.57"	34°27'54.95"	✓	✓	✓	✓	✓	✓	✓	✓
O. karongae	MG KARON1	Mazinzi Bay, Lake Malawi	Malawi	8.58	34.57	✓	✓	✓	✓	✓	✓	✓	✓
O. karongae	MG KARON2	Mazinzi Bay, Lake Malawi	Malawi	8.58	34.57	✓	✓	✓	✓	✓	✓	✓	✓
O. aff korogwe	ZSM 1078	Lake Chala	Tanzania	-	-	✓	✓	-	–	–	–	–	-
O. korogwe	MG P4A8	Zigi river	Tanzania	5.12	38.86	✓	✓	✓	✓	✓	✓	✓	✓
O. korogwe	MG P4A9	Zigi river	Tanzania	5.12	38.86	✓	✓	✓	✓	✓	✓	✓	✓
O. korogwe	MG P4C7	Zigi river	Tanzania	5.04	38.90	✓	✓	✓	✓	✓	✓	✓	✓
O. korogwe	MG P4C8	Zigi river	Tanzania	5.04	38.90	✓	✓	✓	x	✓	✓	✓	✓
O. lepidurus	AMNH 263330	Congo River	DRC	5.91	12.76	✓	✓	✓	✓	✓	✓	✓	✓
O. lepidurus	AMNH 263633	Congo River	DRC	5.85	13.04	✓	✓	✓	✓	✓	✓	✓	✓
O. leucostictus	MG 246	Songea, fish ponds		10.37	35.38	✓	✓	✓	✓	✓	✓	x	✓
O. leucostictus	MG 247	Songea, fish ponds		10.37	35.38	✓	✓	✓	x	x	✓	✓	✓
O. leucostictus	RMCA 3758	Ituri River, Ituri Basin	DRC	-	-	✓	✓	✓	✓	✓	✓	✓	✓
O. leucostictus	RMCA 3759	Ituri River, Ituri Basin	DRC	-	-	✓	✓	✓	-	✓	✓	✓	✓
O. leucostictus	AF 042	Fish pond, edge of Lake Eyasi	Tanzania	3.425306 S	35.343723 E	✓	✓	✓	✓	✓	✓	✓	✓

O. macrochir	SAIAB 130	Cunene Lagoon	Namibia	-17.26	11.76	✓	✓	✓	✓	✓	✓	✓	✓
O. macrochir	SAIAB 136	Cunene Lagoon	Namibia	-17.26	11.76	✓	✓	✓	✓	✓	✓	✓	✓
O. macrochir	SAIAB RB10-B137	Kabompo River	Zambia	-12.36	25.09	✓	✓	✓	✓	✓	✓	✓	✓
O. macrochir	ZSM DRC-2012-1073	River Lufira	DRC	09° 31' 02,2" S	27° 02' 24,6" E	✓	✓	-	-	-	-	-	-
O. malagarasi	MG130	Malagarasi River at Uvinza	Tanzania	5°06.752	30°23.472	✓	✓	✓	✓	✓	✓	✓	✓
O. malagarasi	MG131	Malagarasi River at Uvinza	Tanzania	5°06.752	30°23.472	✓	✓	✓	✓	✓	-	✓	✓
O. malagarasi	MG132	Malagarasi River at Uvinza	Tanzania	5°06.752	30°23.472	✓	✓	✓	✓	✓	✓	✓	✓
O. mortimeri	SAIAB 236	Cahora Bassa	Mozambique	-15.66	30.95	✓	✓	✓	x	✓	✓	✓	✓
O. mortimeri	SAIAB 350	Zambezi River	Mozambique	-15.61	32.67	✓	✓	✓	x	✓	✓	✓	✓
O. mossambicus	SAIAB 036	Fish River	Namibia	-24.49	17.85	✓	✓	✓	x	✓	✓	✓	✓
O. mossambicus	SAIAB M1	Changane River	Mozambique	-22.94	33.67	✓	✓	✓	x	✓	✓	✓	✓
O. mossambicus	SAIAB NJ10-A087	Limpopo River	South Africa	-23.99	31.82	✓	✓	✓	x	✓	✓	✓	✓
O. mossambicus	SAIAB SA9	Luvhuvu River	South Africa	-23.11	30.12	✓	✓	✓	x	✓	✓	x	✓
O. mweruensis	OS IK16-1	Kashikishi	Zambia	9°18'53.85"	28°43'50.25"	✓	✓	✓	✓	✓	✓	✓	✓
O. mweruensis	OS IK16-4	Kashikishi	Zambia	9°18'53.85"	28°43'50.25"	✓	✓	✓	✓	✓	✓	✓	✓
O. niloticus	MG 277A	Lake Itamba	Tanzania	9.21	33.50	✓	✓	-	-	-	-	-	-
O. niloticus	SAIAB 382	Zambezi River	Mozambique	-15.61	32.67	✓	✓	✓	✓	✓	✓	✓	x
O. niloticus cancellatus	EB A544	Awash	Ethiopia	-	-	✓	✓	✓	✓	✓	✓	✓	-
O. niloticus filoa	EB A572	Awash	Ethiopia	-	-	✓	✓	✓	✓	✓	✓	✓	✓
O. niloticus niloticus	EB A056	-	Ghana	-	-	✓	✓	✓	✓	✓	✓	✓	✓
O. niloticus	AF 007	Lake Manyara	Tanzania	-3.42	35.85	✓	✓	✓	✓	✓	✓	✓	✓
O. placidus placidus	SAIAB RC10 C072	Zambezi River	Mozambique	-16.31	33.73	✓	✓	✓	✓	✓	✓	✓	✓
O. placidus placidus	SAIAB RC10-C078	Zambezi River	Mozambique	-16.31	33.73	✓	✓	✓	✓	✓	✓	✓	✓
O. placidus “ruvumae”	MG 102	Rovuma River	Tanzania	11.24	38.29	✓	✓	✓	✓	✓	✓	✓	✓
O. placidus “ruvumae”	MG 103	Rovuma River	Tanzania	11.24	38.29	✓	✓	✓	✓	✓	✓	✓	✓

O. placidus "ruvumae"	SAIAB N126	Rovuma River	Mozambique	-11.43	38.48	✓	✓	✓	✓	✓	✓	✓	✓
O. placidus "ruvumae"	SAIAB N299	Lugenda River	Mozambique	-12.18	38.08	✓	✓	✓	✓	✓	✓	✓	✓
O. rukwaensis	MG 67A	Lake Rukwa	Tanzania	8.23	32.54	✓	✓	✓	✓	✓	✓	✓	✓
O. rukwaensis	MG 67B	Lake Rukwa	Tanzania	8.23	32.54	✓	✓	✓	✓	✓	✓	✓	✓
O. variabilis	MG395	Makobe Island	Tanzania	2°21'56.15"	32°55'21.19"	✓	✓	✓	✓	✓	✓	✓	✓
O. variabilis	MG396	Makobe Island	Tanzania	2°21'56.15"	32°55'21.19"	✓	✓	✓	✓	✓	✓	✓	✓
O. variabilis	MG397	Makobe Island	Tanzania	2°21'56.15"	32°55'21.19"	✓	✓	✓	✓	✓	✓	✓	✓
O. salinicola	ZSM ULI-005	Saline swamp near Mwashia village	DRC	10.70	27.34	✓	✓	✓	x	✓	✓	✓	✓
O. schwebischi	ZSM P AA 727	Ivindo River	Gabon	0.55	12.86	✓	✓	✓	✓	✓	✓	✓	✓
O. schwebischi	ZSM P AA 0930	Ivindo River	Gabon	0.55	12.86	✓	✓	✓	✓	✓	✓	✓	✓
O. schwebischi	ZSM P AA 0931	Ivindo River	Gabon	0.55	12.86	✓	✓	✓	✓	✓	✓	✓	✓
O. shiranus chilwae	MG 298A	Lake Chilwa	Malawi	15.22	35.34	✓	✓	✓	✓	✓	✓	✓	✓
O. shiranus shiranus	MG 408A	Lake Malawi (Salima market)	Malawi	-	-	✓	X	✓	✓	✓	✓	✓	✓
O. shiranus shiranus	SAIAB MA11-192	Lake Malawi	Malawi	-12.83	34.16	✓	✓	✓	✓	✓	x	✓	✓
O. shiranus shiranus	SAIAB TM11-198	Lake Malawi	Malawi	-15.80	35.64	✓	✓	✓	✓	✓	✓	✓	✓
O. sp. "rutamba"	MG T3G9	Lake Rutamba	Tanzania	10.04	39.47	✓	✓	✓	✓	✓	✓	✓	✓
O. sp. "rutamba"	MG T3H9	Lake Rutamba	Tanzania	10.04	39.47	✓	✓	✓	x	✓	✓	✓	X
O. sp. "rutamba"	MG T3J5	Lake Nambawala	Malawi	10.2	39.27	✓	✓	✓	✓	✓	✓	✓	✓
O. sp. "rutamba"	MG T4B5	Lake Nambawala	Malawi	10.2	39.27	✓	✓	✓	✓	✓	✓	✓	-
O. squamipinnis	MG 1A1A	Lake Malawi	Malawi	9.20	33.45	✓	✓	✓	✓	✓	✓	✓	✓
O. squamipinnis	MG 1A1B	Lake Malawi	Malawi	9.20	33.45	✓	✓	✓	✓	✓	✓	✓	✓
O. tanganicae	MG41	Katanga, Lake Tanganyika	Tanzania	4°54.913	29°36.661	✓	✓	✓	✓	✓	✓	✓	✓
O. tanganicae	MG42	Katanga, Lake Tanganyika	Tanzania	4°54.913	29°36.661	✓	✓	✓	x	✓	✓	✓	✓
O. tanganicae	MG43	Katanga, Lake Tanganyika	Tanzania	4°54.913	29°36.661	✓	✓	✓	✓	✓	✓	✓	✓
O. upembae	ZSM 2012 1621	-	DRC	-	-	✓	✓	✓	✓	✓	✓	✓	✓

O. upembae	ZSM 2012 1637	-	DRC	-	-	✓	✓	✓	✓	x	✓	✓	✓
O. urolepis urolepis	MG 3F4A	Ruvu River	Tanzania	6.41	38.42	✓	✓	✓	✓	x	✓	✓	✓
O. urolepis urolepis	MG 3F4B	Ruvu River	Tanzania	6.41	38.42	✓	✓	✓	✓	✓	✓	✓	✓
Non-*Oreochromis* Orochromini													
Konia eisentrauti						AJ845102							
Sarotherodon Barombi Mbo						KJ955426							
Sarotherodon caroli						AJ845112							
Sarotherodon caudomarginatus						AF317243							
Sarotherodon galilaeus	GT U1B1	Lake Albert	Uganda	2.23	31.32	✓	✓	✓	✓	x	✓	✓	x
Sarotherodon linnellii						AJ845114							
Sarotherodon lohbergeri						AJ845108							
Sarotherodon melanotheron						AF317245							
Sarotherodon mvogoi	ZSM PAA600	Ngoila	Cameroon	2.88	13.91	✓	✓	✓	✓	✓	✓	✓	x
Sarotherodon steinbachi						AJ845110							
Sarotherodon occidentalis						AF317246							
Stomatepia mariae						AF317279							
Tristramella simonis						AF317276							
Non-Oreochromini outgroup													
Coptodon cf. rendali	(GT) T7 E10	Lake Nala	Tanzania	-6.945	36.935	✓	✓	✓	✓	✓	✓	x	✓
Coptodon cf. rendali	(GT) T7 F4	Lake Nala	Tanzania	-6.945	36.935	✓	✓	✓	✓	✓	x	x	x
Coptodon sp.	SAIAB TM11-267	Lake Malawi	Malawi	-15.06	35.22	✓	✓	✓	✓	✓	✓	✓	✓

Samples held in national collections: AMNH (American Museum of Natural History); RMCA (Royal Museum for Central Africa); SAIAB (South African Institute of Aquatic Biodiversity); ZSM (Zoologische Staatssammlung München). Personal samples: AF (Antonia Ford, samples held at the Day lab, UCL); MG (Martin Genner, samples held at University of Bristol); GT (George Turner, samples held at Bangor University); EB (Etienne Bezault); OS (Ole Seehausen, samples held at University of Bern).

Grey shaded boxes denote samples downloaded from Genbank (ND2 data only).

Table S2. Primers and reaction conditions for each molecular marker.

Gene	Primers	Primer Sequence	Thermal cycling programme	References
ND2	ND2 H498 ND2 L4299	5'-CGSAGTTGTGTTTGRTT-3' 5'-AAGGRCCACTTTGATAGAGT-3'	94°C, 2 min (94°C, 20 sec 50°C, 20 sec, 72°C, 1 min) x32 72°C, 7 min	Macey et. al. 1997 Hrbek & Larson, 1999
ND2	ND2 –OHE ND2-OLI	5'-CCCTGATTCTCCAAATCCAA-3' 5'-ATTTTCACTCCCGCTTAGGG-3'	94°C, 2 min (94°C, 20 sec 48°C, 20 sec 72°C, 1 min) x32 72°C, 7 min	This study
16S	16sar-L 16sbr-H	5'-CGCCTGTTTATCAAAAACAT-3' 5'-CCGGTCTGAACTCAGATCA-3'	95°C, 1 min (95°C, 30 sec 48°C, 30 sec 72°C, 1 min) x34 72°C, 5 min	Palumbi et al., 1991
S7	S7 1F S7 2R	5'-TGGCCTCTTCCTTGGCCGTC-3' 5'-AACTCGTCTGGCTTTTCGCC-3'	95°C, 1 min (95°C, 1 min 56°C, 1 min 72°C, 90 sec) x30 72°C, 10 min	Chow & Hazama, 1998
Bmp4	Bmp4-F Bmp4-R	5'-GAGGACCCATGCCCATTCG-3' 5'-GCCACTATCCAGTCATTCC-3'	94°C, 2 min (94°C, 30 sec 62°C, 30 sec 72°C, 1 min) x32 72°C, 10 min	Salzburger & Meyer, 2012
Gapdhs	Gapdhs-F Gapdhs-R	5'-CCCTGGCCAAAGTCATCCACGATA-3' 5'-CACCACTGACACATCGGCC-3'	94°C, 2 min (94°C, 30 sec 60°C, 30 sec 72°C, 1 min) x32 72°C, 10 min	Salzburger & Meyer, 2012
Tyr	Tyr-F Tyr-R	5'-TGGGTGGACGCAACTCCCTT-3' 5'-TGGCAAATCGGTCCATGGGT-3'	94°C, 2 min (94°C, 30 sec 60°C, 30 sec 72°C, 1 min) x32 72°C, 10 min	Salzburger & Meyer, 2012
Ccng1	ng1-F Ccng1-R	5'-CTGCTTGCCCTGGCTCTCCT-3' 5'-AGCTGACTCAGGTATGGTCGGA-3'	94°C, 2 min (94°C, 30 sec 58°C, 30 sec 72°C, 1 min) x32 72°C, 10 min	Salzburger & Meyer, 2012

References

Chow S, Hazama K (1998) Universal PCR primers for S7 ribosomal protein gene introns in fish. *Molecular Ecology* **7**, 1255-1256.

Hrbek T, Larson A (1999) The evolution of diapause in the killifish family Rivulidae (Atherinomorpha, Cyprinodontiformes): a molecular phylogenetic and biogeographic perspective. *Evolution* **53**, 1200-1216.

Macey JR, Larson A, Ananjeva, NB, Fang Z, Papenfuss TJ (1997) Two novel gene orders and the role of light-strandreplication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* **14**, 91-104.

Palumbi S (1991) The simple fool's guide to PCR: version 2.0, Saturday, July 27, 1991. University of Hawaii.

Salzburger W, Meyer BS (2012) A novel primer set for multilocus phylogenetic inference in East African cichlid fishes. *Molecular Ecology Resources* **12**, 1097-1104.

Table S3. Environmental adaptation (tolerance) and morphological traits considered in ancestral state reconstruction analyses.

Species	MrBayes pruned tree	*BEAST species tree	Increased saline tolerance*	Increased thermal tolerance*	Increased pH tolerance	Soda adaptation ^	Genital tassel+^	Extended male jaw +^
A. alcalica	Aalcalica_AF881_19	Aalcalica	1	1	1	1	0	0
A. grahami	Agrahami_AF856_18	Agrahami	1	1	1	1	0	0
A. latilabris	Alatilabris_AF122_05	Alatilabris	1	1	1	1	0	0
A. ndalalani	Andalalani_AF123_05	Andalalani	1	1	1	1	0	0
O. amphimelas	Oamphimelas_AF001_02	Oamphimelas	1	1	1	1	0	0
O. andersonii	Oandersonii_SAIAB_C5	Oandersonii	0	0	0	0	0	0
O. angolensis	Oangolensis_SAIAB_F203	Oangolensis	0	0	0	0	0	0
O. chungruruensis	Ochungruruensis_MG_3D2A	Ochungruruensis	0	0	0	0	1	0
O. esculentus	Oesculentus_MG_21A	Oesculentus	0	0	0	0	0	0
O. hunteri	Ohunteri_MG111333	Ohunteri	0	0	0	0	0	0
O. jipe	Ojipe_pan_MG_P2F2	Ojipe	0	0	0	0	0	1
O. karomo	Okaromo_MG206	Okaromo	0	0	0	0	1	0
O. karongae	Okarongae_MG_226	Okarongae	0	0	0	0	1	0
O. korogwe	Okorogwe_MG_P4A8	Okorogwe	0	0	0	0	0	0
O. lepidurus	Olepidurus_AMNH263330	Olepidurus	0	0	0	0	1	0
O. leucostictus	Oleucostictus_RMCA_3758	Oleucostictus	0	1	0	0	0	0
O. macrochir	Omacrochir_SAIAB130	Omacrochir	0	0	0	0	1	0
O. malagarasi	Omalagarasi_MG130	Omalagarasi	0	0	0	0	1	0
O. mortimeri	Omortimeri_SAIAB236	Omortimeri	0	0	0	0	0	0
O. mossambicus	Omossambicus_SAIAB036	Omossambicus	1	0	0	0	0	1
O.mweruensis	Omweruensis_OS_IK16_1	Omweruensis	0	0	0	0	0	0
O. niloticus niloticus	Oniloticus_AF007_02	Oniloticus	0	0	0	0	0	0
O. niloticus cancellatus	Oniloticus_cancellatus_EBA544	-	0	0	0	0	0	0

O. niloticus filoa	Oniloticus_filoa_EBA572	-	0	1	0	0	0	0
O. placidus placidus	Oplacidus_SAIAB_RC10_C072	Oplacidus	0	0	0	0	0	0
O. placidus ruvumae	Oplacidus_ruvumae_MG102	Oplacidusruvumae	0	0	0	0	0	0
O. rukwaensis	Orukwaensis_MG67A	Orukwaensis	0	0	0	0	1	0
O. sp rutamba	Osp_rutamba_MG_T4B5	Osprutamba	0	0	0	0	0	0
O. variabilis	Ovariabilis_MG395	Ovariabilis	0	0	0	0	1	0
O. salinicola	Osalinicola_ZSM_ULI_005	Osalinicola	1	0	1	0	1	0
O. schwebischi	Oschwebischi_ZSM_P_AA_727	Oschwebischi	0	0	0	0	1	0
O. shiranus	Oshiranus_shiranus_MG408A	Oshiranus	0	0	0	0	0	1
O. shiranus chilwae	Oshiranus_chilwae_MG298A	-	0	1	0	0	0	1
O. squamipinnis	Osquamipinnis_MG1A1A	Osquamipinnis	0	0	0	0	1	0
O. tanganicae	Otanganicae_MG41	Otanganicae	0	0	0	0	0	0
O. upembae	Oupembae_ZSM_2012_1621	Oupembae	0	0	0	0	1	0
O. urolepis	Ourolepis_MG3F4B	Ourolepis	1	1	0	0	0	1
S. galilaeus	Sgalilaeus_U1B1	Sgalilaeus	0	0	0	0	0	0
S. mvogoi	Smvogoi_ZSM_PAA_600	Smvogoi	0	0	0	0	0	0
C. rendalli	Coptodon_rendalli_T7_F4	-	0	0	0	0	0	0

Coding used for BayesTraits analyses
* Tested for correlated trait shifts
+ Tested for correlated trait shifts
^ Trait used (independently) for ancestral state reconstruction
Coding based on data in Table 1 (tolerance traits) and Trewavas 1983 (morphological traits)

Figure S1. Bayesian phylogeny of *Oreochromis* based on concatenated nuclear data (3092 bp, 101 samples). Support values shown below nodes are Bayesian Posterior Probabilities (BPP). Those above the nodes are bootstrap (BS) values generated using Maximum Likelihood. *Coptodon* spp. samples [outgroup] are removed from the figure.

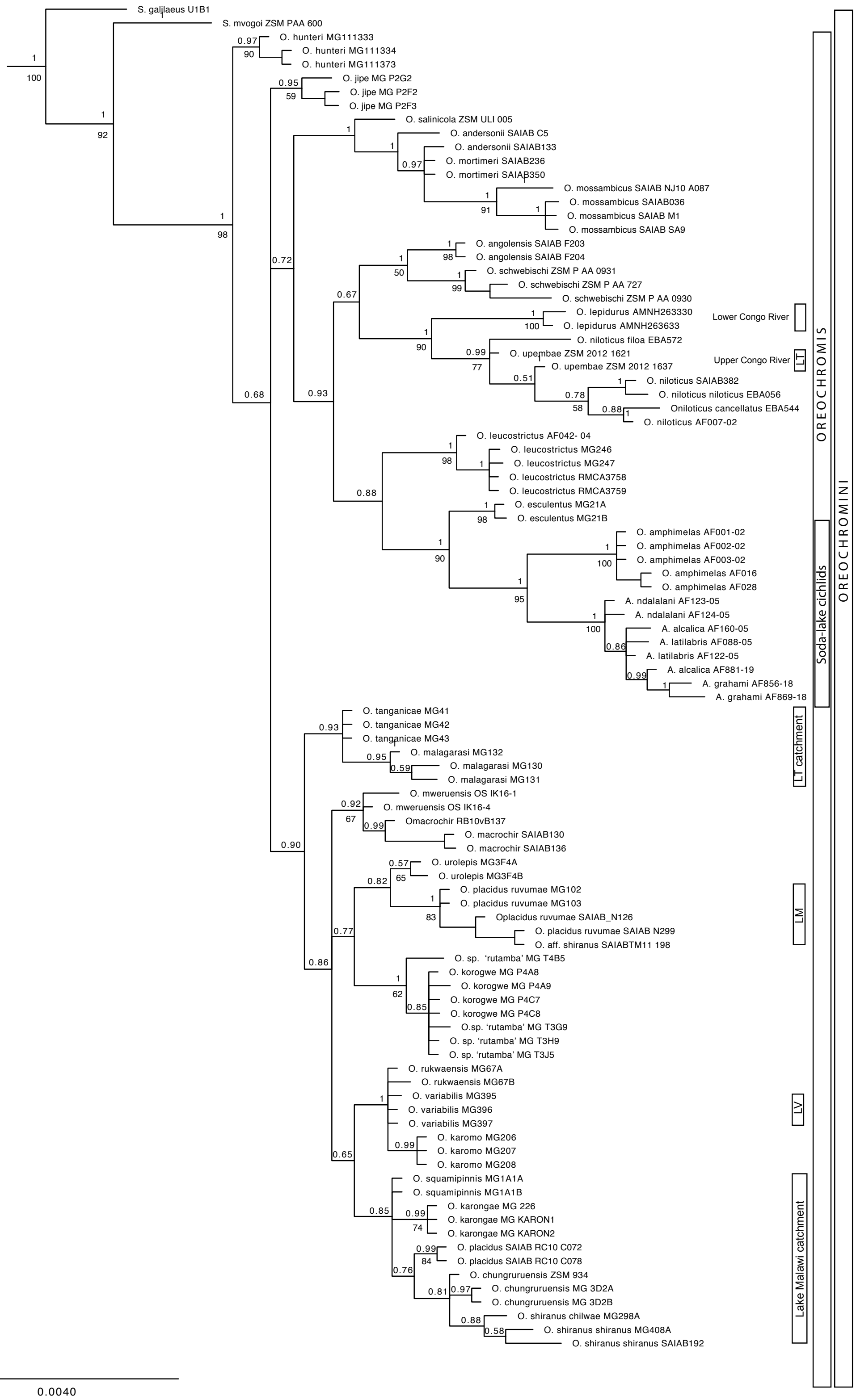

Figure S2. Bayesian phylogeny of *Oreochromis* based on concatenated mtDNA data (1582bp, 116 samples). The tree depicted here includes additional sampling of other Oreochromini taxa. Support values shown below nodes are Bayesian Posterior Probabilities (BPP). Those above the nodes are bootstrap (BS) values generated using Maximum Likelihood. *Coptodon* spp. samples [outgroup] are removed from the figure.

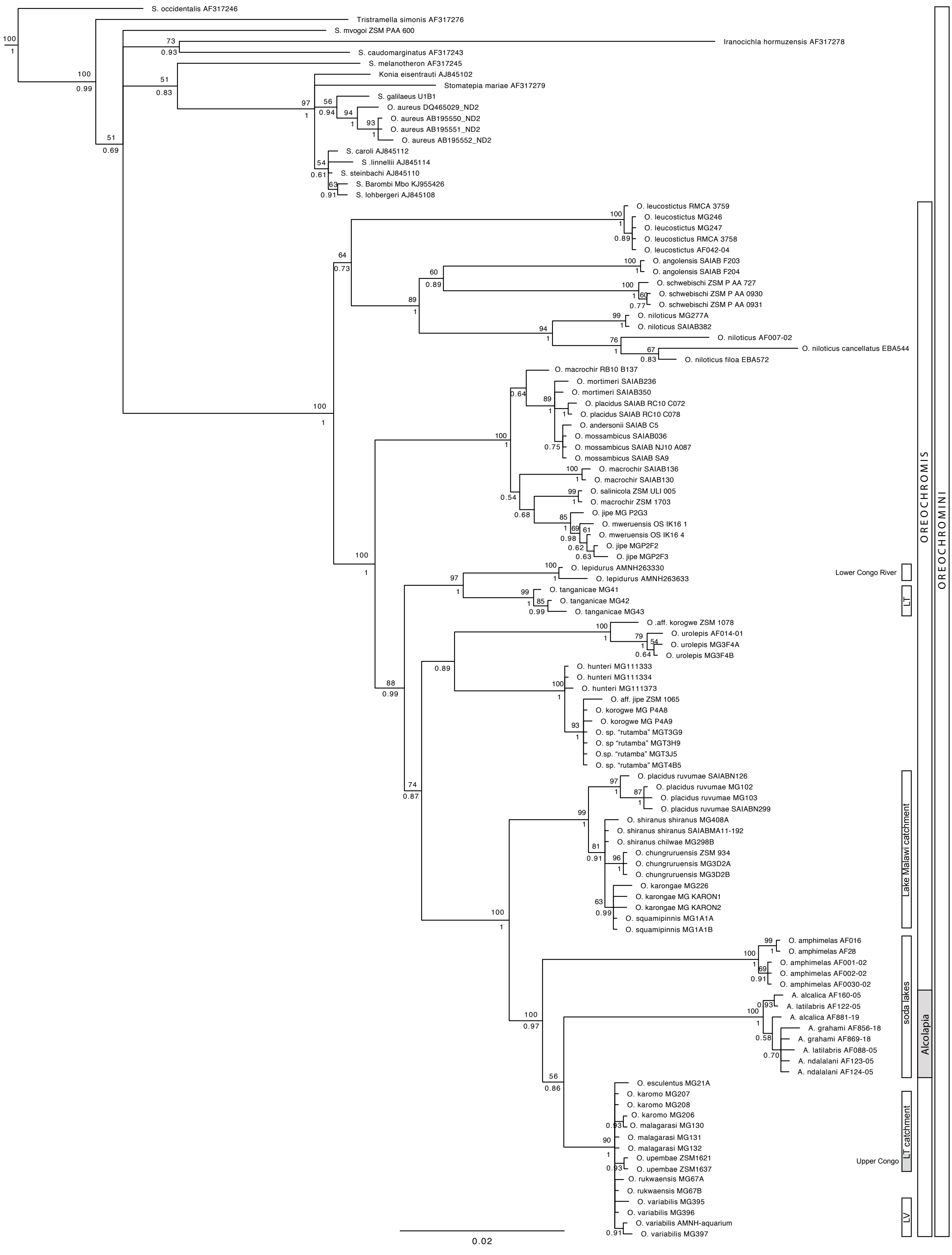

Figure S3. Species tree based on nuclear data and generated using BPP. Support values are shown above branches.

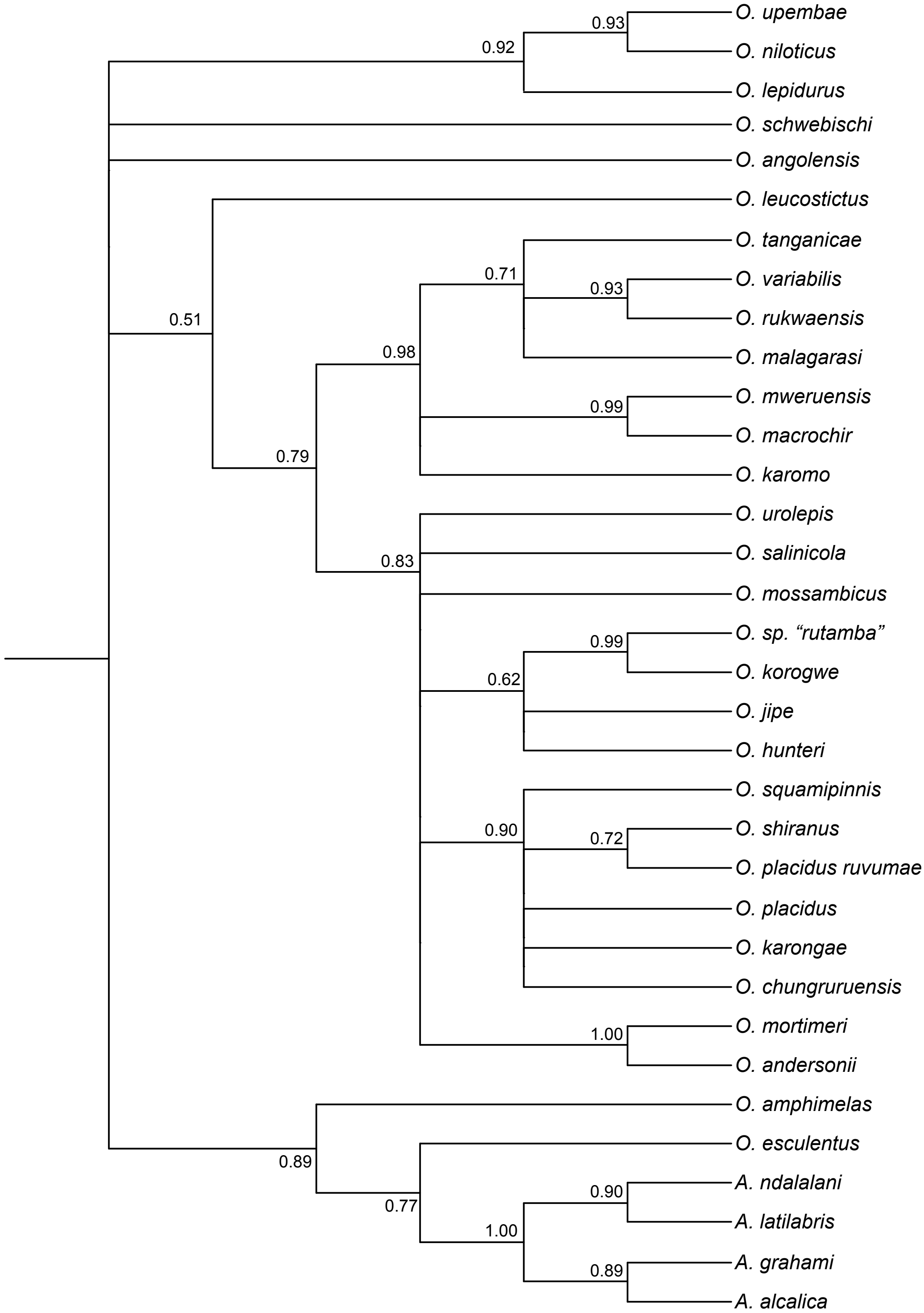

Figure S4. Ancestral state reconstruction from BayesTraits analysis based on the Mr Bayes nuclear concatenated phylogeny. A) Ancestral state reconstruction of thermal/salinity tolerance (TS) and soda adaptation (So). B) Ancestral state reconstruction of phenotypic male secondary sexual characteristics: genital tassel (GT) and extended jaw morphology (EJ). Colour coding are as per Figure 2.

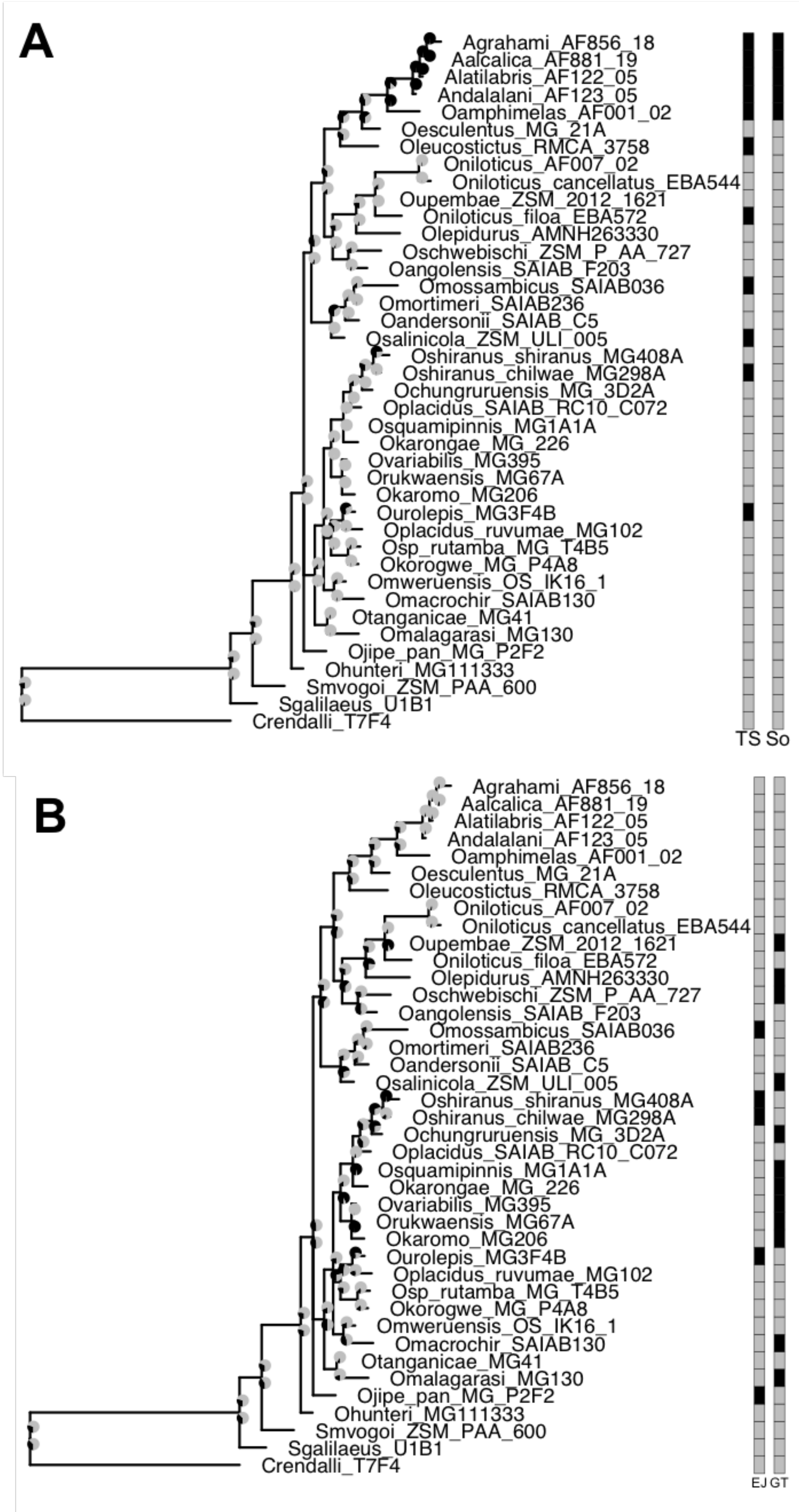

Figure S5. Ancestral state reconstructions of environmental tolerances as independent traits. A) Reconstruction using the *BEAST species trees; B) reconstruction using the MrBayes concatenated nuclear phylogeny.