Rapid Parallel Morphological and Mechanical Diversification of South American Pike Cichlids (*Crenicichla*)

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Supplementary Materials

EXTENDED MATERIAL AND METHODS

RAD library preparation

DNA extraction and ddRAD (“Double-Digest Restriction Site Associated DNA”; Peterson et al. 2012) library preparation as well as the basic bioinformatic processing followed procedures detailed in Piálek et al. (2019). Genomic DNA was extracted from ethanol-preserved gill or fin tissue using the JETQUICK Tissue DNA Spin Kit (Genomed) following standard protocol. 300 ng of genomic DNA from each sample was digested with two restriction enzymes, SphI and MluCI (New England BioLabs, NEB) in one 30 µl reaction (0.25 µl of SphI-HF 20 kU/ml, 2.0 µl of MluCI 10 kU/ml, 3.0 µl of NEBuffer 4) for 3 h at 37 °C. Digestion products were purified with AMPure XP beads (Beckman Coulter) with a ratio of beads/product volume set to 1.5, eluted into 40 µl of 1x TE buffer, and quantified. P1 and P2 ‘‘flex’’ adapters (*sensu* Peterson et al., 2012) were ligated in a 40 µl reaction with 100 ng of the purified digestion product (0.1 µl of NEB T4 DNA Ligase 400 kU/ml, 4 µl of 109 T4 buffer, 1.6 µl of P1 adapter 0.5 µM, 0.12 µl of P2 adapter 100 µM) performed in a thermal cycler (ligation 23 °C, 30 min; ligase deactivation 65 °C, 10 min; slow cooling 1 °C/45 s). The total volume of each 48 ligation products differing in adapter barcode were pooled together (into a ‘‘sublibrary’’) and cleaned with AMPure XP beads (1.5 ratio) in a twostep procedure enabling final elution into 33 µl volume (1x TE buffer); the order of samples was randomized between and within sublibraries. Automated size selection of a fraction of 276–324 bp separately from each sublibrary was performed on Pippin Prep system (Sage Science) with CSD2010 kit. PCR amplification with primers bearing the multiplexing indices and Illumina flow cell annealing regions was done in 4 to 6 independent 50 µl reactions for every sublibrary, each reaction containing 10 ng of separated DNA, 1 µl of each primer 25 µM, 1 µl of Herculase II Fusion DNA Polymerase (Agilent), 10 µl of 5x Herculase II Reaction Buffer, and 0.5 µl of dNTPs 100 mM: 98 °C, 2 min; 10x [98 °C, 30 s; 65 °C, 30 s; 72 °C, 1 min]; 72 °C, 5 min; hold on 10 °C. PCR products were purified on AMPure XP beads and combined in equimolar ratios to compose a final library. Sequencing was performed on an Illumina HiSeq 4000 (150 cycles P/E) in the EMBL Genomic Core Facility, Heidelberg, Germany.

Delineation of feeding ecologies

We classified species into five feeding ecologies based on a combination of preliminary gut content analyses and a detailed assessment of the dentition of the lower pharyngeal jaw. Prey were classified into five broad categories following Burress et al. (2013): fish, microinvertebrates (*e.g.*, small aquatic larvae and nymphs), macroinvertebrates (*e.g.*, shrimps and crabs), mollusks (*e.g.*, snails and bivalves), and algae (*e.g.*, attached filamentous, biofilm, and other vegetative debris).

The pharyngeal jaws are anatomically, functionally, and evolutionarily decoupled from the oral jaws (Liem 1973; Hulsey et al. 2006; Burress et al. 2020) and are responsible for processing (*i.e.*, grasping, crushing, tearing, etc.) prey in ray-finned fishes (Liem 1973; Lauder 1982; 1983). Pharyngeal jaw dentition is known to closely reflect feeding ecology in cichlids (Burress 2016) and *Crenicichla* specifically (Burress et al. 2013; 2016); therefore, we focused on the presence and relative frequency of major tooth types following Casciotta and Arratia (1993): (i) unicuspid, recurved, (ii) generic bicuspid, (iii) molariform, (iv) bevelled, (v) generic unicuspid, conical, (vi) bicuspid, recurved, and (vii) bicuspid, crenulate (illustrated in Burress 2016).

We used gut contents and pharyngeal jaw dentition in combination to classify species into five general feeding guilds: piscivore, macroinvertivore, molluscivore, browser, and crevice-feeder. Piscivores eat almost exclusively fishes and possess largely simple and recurved conical pharyngeal teeth (1-2 tooth types). Macroinvertivores principally eat crabs, shrimps, and large aquatic insects (*e.g.*, dragonflies), but may supplement these with small fractions of fish and molluscs. Macroinvertivores have diverse pharyngeal teeth (4-6 tooth types), including conical, crenulate, and bevelled tooth types. Molluscivores principally eat snails and bivalves, but may secondarily eat small aquatic insects (*e.g.*, trichopterans, chironomids, and dipterans) and possess enlarged molariform pharyngeal teeth surrounded by few simple conical teeth (1-2 tooth types). Crevice feeders almost exclusively eat rock-clinging aquatic insects (*e.g.*, ephemeropterans and plecopterans). Browsers eat filamentous algae, biofilm, and small rock-associated aquatic insects (*e.g.*, trichopterans and ephemeropterans). Crevice feeders and browsers have similar pharyngeal dentition, including 3-4 types, but not recurved conical or molariform teeth.

Supplementary Tables and Figures

Table S1. Sampling for the phylogenetic portion of the study.

label	Species	Locale	Country
C1214_missioneira_UYaboti1	Crenicichla_missioneira	Rio Yaboti	Argentina
C1215_missioneira_UYaboti2	Crenicichla_missioneira	Rio Yaboti	Argentina
C1235_missioneira_UYaboti3	Crenicichla_missioneira	Rio Yaboti	Argentina
C1237_missioneira_UYaboti4	Crenicichla_missioneira	Rio Yaboti	Argentina
C1290_missioneira_LParaiso1	Crenicichla_missioneira	Rio Paraiso	Argentina
C1297_missioneira_LParaiso2	Crenicichla_missioneira	Rio Paraiso	Argentina
C1303_missioneira_LParaiso3	Crenicichla_missioneira	Rio Paraiso	Argentina
C1278_missioneira_MSoberbio2	Crenicichla_missioneira	Rio Soberbio	Argentina
C1268_missioneira_MSoberbio1	Crenicichla_missioneira	Rio Soberbio	Argentina
C1096_missioneira_Queguay3	Crenicichla_missioneira	Rio Queguay	Uruguay
C1095_missioneira_Queguay2	Crenicichla_missioneira	Rio Queguay	Uruguay
C1099_missioneira_Uruguay	Crenicichla_missioneira	Rio Uruguay	Uruguay
C1087_missioneira_Catalan	Crenicichla_missioneira	Arroyo Catalan Grande	Uruguay
C1091_missioneira_Negro	Crenicichla_missioneira	Rio Negro	Uruguay
C1101_missioneira_Yi	Crenicichla_missioneira	Rio Yi	Uruguay
C1097_missioneira_Tacuarembo	Crenicichla_missioneira	Rio Tacuarembo	Uruguay
C1298_missioneiraLIPS_LParaiso1	Crenicichla_cf_missioneira	Rio Paraiso	Argentina
C1300_missioneiraLIPS_LParaiso2	Crenicichla_cf_missioneira	Rio Paraiso	Argentina
C1217_tendybaguassu_UYaboti2	Crenicichla_tendybaguassu	Rio Yaboti	Argentina
C1216_tendybaguassu_UYaboti1	Crenicichla_tendybaguassu	Rio Yaboti	Argentina
C1287_missioneiraLIPS_MSoberbio2	Crenicichla_cf_missioneira	Rio Soberbio	Argentina
C1284_missioneiraLIPS_MSoberbio1	Crenicichla_cf_missioneira	Rio Soberbio	Argentina
C1265_tendybaguassu_MSoberbio1	Crenicichla_tendybaguassu	Rio Soberbio	Argentina
C1280_tendybaguassu_MSoberbio3	Crenicichla_tendybaguassu	Rio Soberbio	Argentina
C1266_tendybaguassu_MSoberbio2	Crenicichla_tendybaguassu	Rio Soberbio	Argentina
C1113_tendybaguassu_Cuareim2	Crenicichla_tendybaguassu	Rio Cuareim	Uruguay
C1112_tendybaguassu_Cuareim1	Crenicichla_tendybaguassu	Rio Cuareim	Uruguay
C1405_missioneira_Toro4	Crenicichla_missioneira	Arroyo_Toro	Argentina
C1370_missioneira_Toro1	Crenicichla_missioneira	Arroyo_Toro	Argentina
C1399_missioneira_Toro3	Crenicichla_missioneira	Arroyo_Toro	Argentina
C1389_missioneira_Toro2	Crenicichla_missioneira	Arroyo_Toro	Argentina
C1404_tendybaguassu_Toro4	Crenicichla_tendybaguassu	Arroyo_Toro	Argentina
C1400_tendybaguassu_Toro3	Crenicichla_tendybaguassu	Arroyo_Toro	Argentina
C1396_tendybaguassu_Toro2	Crenicichla_tendybaguassu	Arroyo_Toro	Argentina
C1371_tendybaguassu_Toro1	Crenicichla_tendybaguassu	Arroyo_Toro	Argentina
C1079_minuano_Negro	Crenicichla_minuano	Rio Negro	Uruguay
C1392_minuanoNEW_Toro2	Crenicichla_minuano	Arroyo_Toro	Argentina

C1390_minuanoNEW_Toro1	Crenicichla_minuano	Arroyo_Toro	Argentina
C126_celidochilus_Jacutinga	Crenicichla_celidochilus	Rio Jacuntinga	Brazil
C1069_celidochilus_Catalan1	Crenicichla_celidochilus	Arroyo Catalan Grande	Uruguay
C1210_minuanoNEW_UYaboti2	Crenicichla_cf_minuano	Rio Yaboti	Argentina
C1220_minuanoNEW_UYaboti3	Crenicichla_cf_minuano	Rio Yaboti	Argentina
C1208_minuanoNEW_UYaboti1	Crenicichla_cf_minuano	Rio Yaboti	Argentina
C1292_minuanoNEW_LParaiso1	Crenicichla_cf_minuano	Rio Paraiso	Argentina
C1302_minuanoNEW_LParaiso2	Crenicichla_cf_minuano	Rio Paraiso	Argentina
C1306_minuanoNEW_LParaiso3	Crenicichla_cf_minuano	Rio Paraiso	Argentina
C1212_hadrostigma_UYaboti1	Crenicichla_hadrostigma	Rio Yaboti	Argentina
C1225_hadrostigma_UYaboti2	Crenicichla_hadrostigma	Rio Yaboti	Argentina
C1229_hadrostigma_UYaboti3	Crenicichla_hadrostigma	Rio Yaboti	Argentina
C1388_minuano_Toro3	Crenicichla_minuano	Arroyo_Toro	Argentina
C1369_minuano_Toro1	Crenicichla_minuano	Arroyo_Toro	Argentina
C1394_minuano_Toro4	Crenicichla_minuano	Arroyo_Toro	Argentina
C1395_minuano_Toro5	Crenicichla_minuano	Arroyo_Toro	Argentina
C1383_minuano_Toro2	Crenicichla_minuano	Arroyo_Toro	Argentina
C1276_minuano_MSoberbio2	Crenicichla_minuano	Rio Soberbio	Argentina
C1296_minuano_cf_hadro_LParaiso	Crenicichla__cf_minuano	Rio Paraiso	Argentina
C1267_minuano_MSoberbio1	Crenicichla_minuano	Rio Soberbio	Argentina
C1288_minuano_LParaiso1	Crenicichla_minuano	Rio Paraiso	Argentina
C1295_minuano_LParaiso3	Crenicichla_minuano	Rio Paraiso	Argentina
C1294_minuano_LParaiso2	Crenicichla_minuano	Rio Paraiso	Argentina
C1219_minuano_UYaboti1	Crenicichla_minuano	Rio Yaboti	Argentina
C1228_minuano_UYaboti3	Crenicichla_minuano	Rio Yaboti	Argentina
C1227_minuano_UYaboti2	Crenicichla_minuano	Rio Yaboti	Argentina
C1070_celidochilus_Catalan2	Crenicichla_celidochilus	Arroyo Catalan Grande	Uruguay
C1094_missioneira_Queguay1	Crenicichla_missioneira	Rio Queguay	Uruguay
C654_macrophthalma	Crenicichla_macrophthalma	Rio Xingu	Brazil
C520_stocki	Crenicichla_stocki	Unknown	Brazil
C262_geayi	Crenicichla_geayi	Rio Bocono	Venezuela
C557_tingui2	Crenicichla_tingui	Rio Cubatoao II	Brazil
C556_tingui1	Crenicichla_tingui	Rio Cubatoao II	Brazil
C559_iguapina2	Crenicichla_iguapina	Rio Jacupiranga	Brazil
C558_iguapina1	Crenicichla_iguapina	Rio Jacupiranga	Brazil
III4_vittata2	Crenicichla_vittata	Asuncion	Paraguay
C62_vittata1	Crenicichla_vittata	Laguna Ibera	Argentina
C1332_PirayGuazu_trib2	Crenicichla_sp	Piray Guazu	Argentina
C437_PirayGuazuLINE7	Crenicichla_sp	Piray Guazu	Argentina
C433_PirayGuazuLINE6	Crenicichla_sp	Piray Guazu	Argentina
C18_PirayGuazuLINE2	Crenicichla_sp	Piray Guazu	Argentina
C429_PirayGuazuLINE4	Crenicichla_sp	Piray Guazu	Argentina
C432_PirayGuazuLINE5	Crenicichla_sp	Piray Guazu	Argentina

C82_PirayGuazuLINE3	Crenicichla_sp	Piray Guazu	Argentina
C16_PirayGuazuLINE1	Crenicichla_sp	Piray Guazu	Argentina
C15_PirayGuazu1	Crenicichla_sp	Piray Guazu	Argentina
C17_PirayGuazu2	Crenicichla_sp	Piray Guazu	Argentina
C1327_PirayGuazu_trib1_2	Crenicichla_sp	Piray Guazu	Argentina
C1326_PirayGuazu_trib1_1	Crenicichla_sp	Piray Guazu	Argentina
C1419_yjhui2	Crenicichla_yjhui	Rio Urugua-i	Argentina
C10_yjhui1	Crenicichla_yjhui	Rio Urugua-i	Argentina
C1412_mandelburgeri4	Crenicichla_mandelburgeri	Aquaray Mini	Argentina
C1411_mandelburgeri3	Crenicichla_mandelburgeri	Aquaray Mini	Argentina
C106_mandelburgeri_LTabay	Crenicichla_mandelburgeri	Tabay Mini	Argentina
C40_ParanayGuazu	Crenicichla_sp	Paranay Guazu	Argentina
C1202_gillmorlisi2	Crenicichla_gillmorlisi	Yguazu Reservoir	Paraguay
C1200_gillmorlisi1	Crenicichla_gillmorlisi	Yguazu Reservoir	Paraguay
C1352_mandelburgeri1	Crenicichla_mandelburgeri	Aguaray Macuco	Argentina
C1358_mandelburgeri2	Crenicichla_mandelburgeri	Aguaray Macuco	Argentina
C1181_AguarayGuazu3	Crenicichla_sp	Aguaray Guazu	Argentina
C1179_AguarayGuazu2	Crenicichla_sp	Aguaray Guazu	Argentina
C1178_AguarayGuazu1	Crenicichla_sp	Aguaray Guazu	Argentina
C1408_hu3	Crenicichla_hu	Piray Mini	Argentina
C1410_hu5	Crenicichla_hu	Piray Mini	Argentina
C1409_hu4	Crenicichla_hu	Piray Mini	Argentina
C13_hu1	Crenicichla_hu	Piray Mini	Argentina
C110_hu2	Crenicichla_hu	Piray Mini	Argentina
C1420_ypo6	Crenicichla_ypo	Rio Urugua-i	Argentina
C1422_ypo8	Crenicichla_ypo	Rio Urugua-i	Argentina
C1417_ypo5	Crenicichla_ypo	Rio Urugua-i	Argentina
C1414_ypo2	Crenicichla_ypo	Rio Urugua-i	Argentina
C1413_ypo1	Crenicichla_ypo	Rio Urugua-i	Argentina
C1421_ypo7	Crenicichla_ypo	Rio Urugua-i	Argentina
C1416_ypo4	Crenicichla_ypo	Rio Urugua-i	Argentina
C1415_ypo3	Crenicichla_ypo	Rio Urugua-i	Argentina
C814_taikyra3	Crenicichla_taikyra	Rio Parana	Argentina
C813_taikyra2	Crenicichla_taikyra	Rio Parana	Argentina
C143_taikyra1	Crenicichla_taikyra	Rio Parana	Argentina
C1193_yaha7	Crenicichla_yaha	Rio Urugua-i	Argentina
C1192_yaha6	Crenicichla_yaha	Rio Urugua-i	Argentina
C1187_yaha2	Crenicichla_yaha	Rio Urugua-i	Argentina
C1186_yaha1	Crenicichla_yaha	Rio Urugua-i	Argentina
C1194_yaha8	Crenicichla_yaha	Rio Urugua-i	Argentina
C1191_yaha5	Crenicichla_yaha	Rio Urugua-i	Argentina
C1189_yaha3	Crenicichla_yaha	Rio Urugua-i	Argentina
C1190_yaha4	Crenicichla_yaha	Rio Urugua-i	Argentina

C1349_tapii3	Crenicichla_tapii	Rio Iguazu	Argentina
C1338_tapii1	Crenicichla_tapii	Rio Iguazu	Argentina
C1343_tapii2	Crenicichla_tapii	Rio Iguazu	Argentina
C1351_tapii4	Crenicichla_tapii	Rio Iguazu	Argentina
C1336_tuca3	Crenicichla_tuca	Rio Iguazu	Argentina
C1335_tuca2	Crenicichla_tuca	Rio Iguazu	Argentina
C1334_tuca1	Crenicichla_tuca	Rio Iguazu	Argentina
C1337_tesay1	Crenicichla_tesay	Rio Iguazu	Argentina
C1344_tesay4	Crenicichla_tesay	Rio Iguazu	Argentina
C1339_tesay2	Crenicichla_tesay	Rio Iguazu	Argentina
C1341_tesay3	Crenicichla_tesay	Rio Iguazu	Argentina
C1340_iguassuensis1	Crenicichla_igassuensis	Rio Iguazu	Argentina
C1350_iguassuensis5	Crenicichla_igassuensis	Rio Iguazu	Argentina
C1342_iguassuensis2	Crenicichla_igassuensis	Rio Iguazu	Argentina
C1345_iguassuensis3	Crenicichla_igassuensis	Rio Iguazu	Argentina
C1347_iguassuensis4	Crenicichla_igassuensis	Rio Iguazu	Argentina
C1108_scottii	Crenicichla_scottii	Arroyo Catalan Grande	Uruguay
C79_gaucho1	Crenicichla_gaucho	Salto Golondrinas	Argentina
C96_gaucho2	Crenicichla_gaucho	Salto Golondrinas	Argentina
C555_maculata2	Crenicichla_maculata	Rio Tres Forquilhas	Brazil
C554_maculata1	Crenicichla_maculata	Rio Tres Forquilhas	Brazil
C1104_punctata	Crenicichla_punctata	Rio Olimar	Uruguay
C553_lucenai2	Crenicichla_lucenai	Rio Jacui	Brazil
C552_lucenai1	Crenicichla_lucenai	Rio Jacui	Brazil

Table S2. Morphological data (by individual) used in the study.

species	SL	Premaxilla	Ascending_process	Mandible	Snout_length	Ecomorph	Prey_mobility
scottii	1.082785	0.173186	0.161368	0.298853	0.227887	Macroinvertivore	Semi_evasive
punctata	1.037426	0.09691	0.117271	0.209515	0.170262	Macroinvertivore	Semi_evasive
punctata	0.951823	-0.03152	0.029384	0.117271	0.09691	Macroinvertivore	Semi_evasive
scottii	1.064458	0.143015	0.139879	0.281033	0.20412	Macroinvertivore	Semi_evasive
gaucho	1.072985	0.060698	0.10721	0.235528	0.225309	Macroinvertivore	Semi_evasive
gaucho	0.970812	-0.03621	0.049218	0.146128	0.10721	Macroinvertivore	Semi_evasive
iguapina	1.1827	0.206826	0.274158	0.378398	0.330414	Macroinvertivore	Semi_evasive
iguapina	0.989895	-0.00436	0.053078	0.143015	0.136721	Macroinvertivore	Semi_evasive
maculata	1.230449	0.232996	0.296665	0.4133	0.367356	Macroinvertivore	Semi_evasive
maculata	0.982723	-0.00877	0.045323	0.130334	0.056905	Macroinvertivore	Semi_evasive
maculata	0.986772	0	0.071882	0.143015	0.120574	Macroinvertivore	Semi_evasive
maculata	1.061075	0.079181	0.139879	0.271842	0.214844	Macroinvertivore	Semi_evasive
maculata	1.000868	-0.00877	0.082785	0.130334	0.143015	Macroinvertivore	Semi_evasive

punctata	0.992111	0.037426	0.056905	0.164353	0.133539	Macroinvertivore	Semi_evasive
punctata	1.147367	0.173186	0.190332	0.285557	0.240549	Macroinvertivore	Semi_evasive
missioneira	1.054613	0.120574	0.318063	0.271842	0.260071	Piscivore	Evasive
missioneira	1.041393	0.113943	0.298853	0.303196	0.240549	Piscivore	Evasive
missioneira	1.037426	0.093422	0.296665	0.271842	0.214844	Piscivore	Evasive
celidochilus	0.993877	0.071882	0.252853	0.187521	0.167317	Piscivore	Evasive
celidochilus	0.970347	0	0.227887	0.149219	0.120574	Piscivore	Evasive
celidochilus	0.937518	-0.04096	0.201397	0.127105	0.100371	Piscivore	Evasive
iguassuensis	1.146438	0.245513	0.342423	0.374748	0.313867	Piscivore	Evasive
iguassuensis	1.149835	0.243038	0.342423	0.361728	0.326336	Piscivore	Evasive
iguassuensis	1.084219	0.149219	0.271842	0.285557	0.245513	Piscivore	Evasive
iguassuensis	1.09237	0.152288	0.30103	0.292256	0.247973	Piscivore	Evasive
iguassuensis	1.045714	0.10721	0.243038	0.245513	0.222716	Piscivore	Evasive
iguassuensis	1.178113	0.264818	0.365488	0.383815	0.342423	Piscivore	Evasive
iguassuensis	1.021189	0.071882	0.190332	0.20412	0.173186	Piscivore	Evasive
iguassuensis	1	0.029384	0.198657	0.152288	0.149219	Piscivore	Evasive
iguassuensis	1.052694	0.146128	0.267172	0.260071	0.222716	Piscivore	Evasive
iguassuensis	1.022016	0.082785	0.193125	0.173186	0.170262	Piscivore	Evasive
ypo	1.141763	0.214844	0.320146	0.30963	0.318063	Piscivore	Evasive
ypo	1.158965	0.230449	0.320146	0.361728	0.340444	Piscivore	Evasive
ypo	1.093071	0.127105	0.260071	0.276462	0.264818	Piscivore	Evasive
ypo	1.049606	0.071882	0.212188	0.212188	0.201397	Piscivore	Evasive
ypo	1.078094	0.139879	0.267172	0.276462	0.243038	Piscivore	Evasive
ypo	1.143327	0.201397	0.32838	0.392697	0.33646	Piscivore	Evasive
ypo	1.150756	0.206826	0.303196	0.359835	0.313867	Piscivore	Evasive
ypo	1.093071	0.143015	0.267172	0.25042	0.243038	Piscivore	Evasive
ypo	1.15412	0.198657	0.326336	0.342423	0.303196	Piscivore	Evasive
ypo	1.181844	0.271842	0.39794	0.356026	0.359835	Piscivore	Evasive
missioneira	1.027757	0.075547	0.222716	0.227887	0.190332	Piscivore	Evasive
missioneira	0.998695	0.049218	0.181844	0.209515	0.164353	Piscivore	Evasive
missioneira	0.936011	-0.00877	0.127105	0.130334	0.089905	Piscivore	Evasive
missioneira	1.067443	0.103804	0.267172	0.283301	0.238046	Piscivore	Evasive
missioneira	1.056142	0.113943	0.247973	0.220108	0.1959	Piscivore	Evasive
missioneira	1.055378	0.133539	0.276462	0.281033	0.25042	Piscivore	Evasive
missioneira	1.01157	0.064458	0.217484	0.212188	0.178977	Piscivore	Evasive
missioneira	1.006466	0.056905	0.243038	0.243038	0.201397	Piscivore	Evasive
minuano	0.982271	-0.05061	0.130334	0.056905	0.149219	Molluscivore	Non_evasive
minuano	0.92993	-0.09151	0.10721	0.045323	0.082785	Molluscivore	Non_evasive
minuano	0.887054	-0.13668	0.064458	-0.00436	0.012837	Molluscivore	Non_evasive
minuano	0.860338	-0.13668	0.033424	-0.07058	0.017033	Molluscivore	Non_evasive
tesay	1.145507	0.184691	0.290035	0.285557	0.313867	Molluscivore	Non_evasive
tesay	1.092018	0.10721	0.217484	0.25042	0.255273	Molluscivore	Non_evasive
tesay	1.081347	0.103804	0.201397	0.184691	0.240549	Molluscivore	Non_evasive

tesay	1.077368	0.09691	0.20412	0.222716	0.247973	Molluscivore	Non_evasive
yaha	1.172895	0.209515	0.311754	0.303196	0.338456	Molluscivore	Non_evasive
yaha	1.150449	0.20412	0.271842	0.276462	0.31597	Molluscivore	Non_evasive
yaha	1.033826	0.053078	0.170262	0.190332	0.20412	Molluscivore	Non_evasive
yaha	0.981819	-0.03621	0.11059	0.068186	0.143015	Molluscivore	Non_evasive
yjhui	1.030195	0.056905	0.240549	0.222716	0.158362	Piscivore	Evasive
yjhui	1.093772	0.120574	0.298853	0.296665	0.257679	Piscivore	Evasive
yjhui	1.125481	0.206826	0.33646	0.30103	0.290035	Piscivore	Evasive
yjhui	1.075912	0.143015	0.285557	0.281033	0.25042	Piscivore	Evasive
yjhui	1.028978	0.082785	0.235528	0.198657	0.1959	Piscivore	Evasive
yjhui	1.004751	0.037426	0.214844	0.187521	0.164353	Piscivore	Evasive
minuano	1.11294	0.130334	0.30963	0.267172	0.298853	Molluscivore	Non_evasive
minuano	1.149219	0.198657	0.348305	0.305351	0.334454	Molluscivore	Non_evasive
minuano	1.057286	0.08636	0.255273	0.178977	0.238046	Molluscivore	Non_evasive
minuano	1.113943	0.139879	0.287802	0.271842	0.30103	Molluscivore	Non_evasive
minuano	1.135451	0.127105	0.294466	0.262451	0.30963	Molluscivore	Non_evasive
minuano	1.051538	0.071882	0.212188	0.193125	0.257679	Molluscivore	Non_evasive
minuano	1.076276	0.100371	0.232996	0.184691	0.255273	Molluscivore	Non_evasive
minuano	1.082067	0.056905	0.245513	0.212188	0.227887	Molluscivore	Non_evasive
minuano	1.047275	0.08636	0.20412	0.184691	0.245513	Molluscivore	Non_evasive
minuano	0.943495	-0.07058	0.089905	0.093422	0.100371	Molluscivore	Non_evasive
minuano	1.11227	0.133539	0.292256	0.25042	0.294466	Molluscivore	Non_evasive
minuano	1.082067	0.127105	0.240549	0.220108	0.252853	Molluscivore	Non_evasive
minuano	1.118265	0.139879	0.278754	0.257679	0.294466	Molluscivore	Non_evasive
minuano	1.069668	0.093422	0.243038	0.240549	0.267172	Molluscivore	Non_evasive
minuano	1.020361	0.060698	0.193125	0.187521	0.214844	Molluscivore	Non_evasive
minuano	0.988559	0.004321	0.167317	0.161368	0.190332	Molluscivore	Non_evasive
minuano	1.03543	0.021189	0.149219	0.149219	0.20412	Molluscivore	Non_evasive
tuca	1.105851	0.225309	0.39794	0.32838	0.392697	Crevice_feeder	Non_evasive
tuca	1.049993	0.167317	0.332438	0.276462	0.303196	Crevice_feeder	Non_evasive
tuca	1.030195	0.10721	0.303196	0.257679	0.274158	Crevice_feeder	Non_evasive
tendybaguassu	1.075912	0.232996	0.376577	0.334454	0.359835	Crevice_feeder	Non_evasive
tendybaguassu	1.037426	0.143015	0.350248	0.278754	0.30963	Crevice_feeder	Non_evasive
tendybaguassu	1.021189	0.117271	0.311754	0.255273	0.271842	Crevice_feeder	Non_evasive
tendybaguassu	1.037426	0.161368	0.338456	0.262451	0.322219	Crevice_feeder	Non_evasive
tendybaguassu	1.064458	0.149219	0.324282	0.307496	0.31597	Crevice_feeder	Non_evasive
tendybaguassu	0.922206	0.037426	0.201397	0.130334	0.152288	Crevice_feeder	Non_evasive
tendybaguassu	1.100026	0.181844	0.371068	0.344392	0.380211	Crevice_feeder	Non_evasive
tendybaguassu	1.067443	0.149219	0.340444	0.307496	0.320146	Crevice_feeder	Non_evasive
tendybaguassu	1.031408	0.117271	0.31597	0.262451	0.281033	Crevice_feeder	Non_evasive
tendybaguassu	1.138934	0.245513	0.396199	0.390935	0.416641	Crevice_feeder	Non_evasive
tendybaguassu	1.144263	0.260071	0.421604	0.414973	0.401401	Crevice_feeder	Non_evasive
tendybaguassu	1.193959	0.313867	0.456366	0.429752	0.447158	Crevice_feeder	Non_evasive

tapii	1.111599	0.021189	0.214844	0.178977	0.262451	Browser	Non_evasive
tapii	1.054996	-0.07572	0.161368	0.120574	0.158362	Browser	Non_evasive
tapii	1.101747	-0.02228	0.158362	0.149219	0.232996	Browser	Non_evasive
tapii	1.046105	-0.10791	0.113943	0.082785	0.170262	Browser	Non_evasive
hadrostigma	1.093422	-0.01773	0.158362	0.176091	0.230449	Browser	Non_evasive
hadrostigma	0.895975	-0.25181	-0.03152	-0.04576	0.021189	Browser	Non_evasive
hadrostigma	0.946943	-0.17393	0.060698	0.029384	0.075547	Browser	Non_evasive
hadrostigma	0.929419	-0.19382	0.021189	0.004321	0.049218	Browser	Non_evasive
hadrostigma	0.889302	-0.29243	-0.04576	-0.09151	-0.05061	Browser	Non_evasive
hadrostigma	0.888741	-0.25181	-0.02228	-0.03621	0.025306	Browser	Non_evasive
tapii	1.103804	-0.00877	0.214844	0.146128	0.227887	Browser	Non_evasive
tapii	1.064458	-0.03621	0.161368	0.11059	0.187521	Browser	Non_evasive
tapii	1.07225	-0.02228	0.120574	0.117271	0.187521	Browser	Non_evasive
tapii	1.051924	-0.04576	0.123852	0.09691	0.20412	Browser	Non_evasive
aquaray_guazu	0.89487	-0.03152	0.127105	0.146128	0.079181	Piscivore	Evasive
aquaray_guazu	1.158362	0.225309	0.394452	0.434569	0.348305	Piscivore	Evasive
aquaray_guazu	1.122544	0.176091	0.389166	0.371068	0.330414	Piscivore	Evasive
gillmorlisi	1.006466	0.025306	0.120574	0.227887	0.149219	Macroinvertivore	Semi_evasive
gillmorlisi	1.026942	0.053078	0.139879	0.245513	0.155336	Macroinvertivore	Semi_evasive
lucenai	0.753583	-0.22915	-0.1549	-0.10791	-0.07058	Macroinvertivore	Semi_evasive
lucenai	0.826075	-0.16749	-0.08619	0.021189	-0.04096	Macroinvertivore	Semi_evasive
piray_guazu	0.897077	-0.07572	0.082785	0.093422	0.120574	Piscivore	Evasive
piray_guazu	0.877947	-0.09151	0.079181	0.064458	0.100371	Piscivore	Evasive
piray_guazu	0.638489	-0.3279	-0.18709	-0.14874	-0.16749	Piscivore	Evasive
piray_guazu	0.954243	-0.02228	0.10721	0.178977	0.143015	Piscivore	Evasive
piray_guazu	0.878522	-0.09151	0.056905	0.082785	0.060698	Piscivore	Evasive
taikyra	1.126456	0.123852	0.235528	0.187521	0.276462	Molluscivore	Non_evasive
taikyra	0.964731	-0.03152	0.075547	0.037426	0.127105	Molluscivore	Non_evasive
taikyra	1.070038	0.056905	0.252853	0.201397	0.269513	Molluscivore	Non_evasive

Footnote: Values are log-transformed. These data were used to visually compare ecomorphs in a non-phylogenetic context (results shown in Figure 1b).

Table S3. Morphological data (by population) used in the study.

x	Premaxilla	Ascending process	Mandible	Snout length	Prey_mobility
AguarayGuazu	0.035628	0.07536	0.089153	0.020673	Evasive
gillmorlisi	0.001042	-0.05305	0.054291	-0.03517	Semi_evasive
yjhui	0.018771	0.03907	0.018464	-0.01384	Evasive
PirayGuazu	0.038008	0.024141	0.054434	0.041927	Evasive
tesay	-0.01235	-0.04337	-0.0364	-0.01055	Non_evasive
tuca	0.074902	0.112641	0.055673	0.087754	Non_evasive
iguassuensis	0.03713	0.021169	0.022681	-0.0125	Evasive
tapii	-0.1453	-0.08809	-0.12163	-0.04642	Non_evasive
taikyra	-0.03219	-0.03504	-0.0807	-0.00245	Non_evasive
yaha	-0.01096	-0.04016	-0.04709	-0.00936	Non_evasive
ypo	0.015058	0.001402	0.01383	-0.00954	Evasive
lucenai	0.031723	-0.06045	0.021722	-0.00213	Semi_evasive
punctata	0.012555	-0.10141	-0.00512	-0.04358	Semi_evasive
maculata	-0.02139	-0.09424	-0.00356	-0.04482	Semi_evasive
gaucho	-0.03205	-0.11064	0.002754	-0.02671	Semi_evasive
scottii	0.052667	-0.09371	0.045445	-0.03193	Semi_evasive
iguapina	-0.01919	-0.09432	0.002381	-0.02783	Semi_evasive
missioneira_Toro	0.036691	0.058368	0.054148	0.006661	Evasive
tendybaguassu_Toro	0.090561	0.123398	0.068435	0.090841	Non_evasive
missioneira_Yaboti	0.029551	0.042799	0.040767	0.001382	Evasive
tendybaguassu_Cuareim	0.059702	0.097382	0.071237	0.085002	Non_evasive
hadrostigma	-0.14515	-0.07823	-0.09337	-0.04809	Non_evasive
minuano_Yaboti	-0.01457	-0.00051	-0.03492	0.003614	Non_evasive
celidochilus_Catalan1	0.030621	0.097151	0.026157	-0.00547	Evasive

Footnote: Values are phylogenetic residuals. These data match the tips of the SNAPP species tree and were used during the phylogenetic ANOVA and MuSSCRat analyses (results shown in Figures 1 and 2).

Table S4. Kinematic data (by individual) used in the study.

	kt	kinesis	ka	Ecomorph
iguassuensis1	0.483254	0.328255	0.073603	Piscivore
iguassuensis2	0.503517	0.325597	0.076972	Piscivore
iguassuensis3	0.585639	0.293774	0.084583	Piscivore
iguassuensis4	0.530835	0.315936	0.082109	Piscivore
iguassuensis5	0.470685	0.325033	0.075002	Piscivore
missioneira1	0.553137	0.325617	0.080348	Piscivore
missioneira2	0.590397	0.330107	0.083317	Piscivore
missioneira3	0.542011	0.317434	0.079313	Piscivore
missioneira4	0.586854	0.316484	0.08229	Piscivore
missioneira5	0.495865	0.295813	0.08196	Piscivore
yjhui1	0.575934	0.294677	0.084991	Piscivore
yjhui2	0.425143	0.260243	0.0858	Piscivore
yjhui3	0.479736	0.291885	0.081734	Piscivore
yjhui4	0.464282	0.245097	0.084174	Piscivore
ypo1	0.436174	0.280626	0.076996	Piscivore
ypo2	0.471522	0.288556	0.081395	Piscivore
ypo3	0.664074	0.344379	0.082505	Piscivore
ypo4	0.475916	0.319812	0.075141	Piscivore
ypo5	0.432565	0.257011	0.085558	Piscivore
gaucho1	0.616985	0.29592	0.085733	Macroinvertivore
iguapina1	0.52095	0.276893	0.084984	Macroinvertivore
iguapina2	0.49335	0.308758	0.075255	Macroinvertivore
maculata1	0.60477	0.327866	0.079865	Macroinvertivore
maculata3	0.678639	0.333922	0.083681	Macroinvertivore
maculata6	0.648855	0.314707	0.08644	Macroinvertivore
punctata1	0.480429	0.326082	0.073634	Macroinvertivore
punctata2	0.566708	0.319439	0.078728	Macroinvertivore
punctata4	0.550212	0.319728	0.077471	Macroinvertivore
scottii1	0.479379	0.319392	0.073823	Macroinvertivore
tendybaguassu1	0.471778	0.23908	0.089181	Crevice_feeder
tendybaguassu2	0.458584	0.232943	0.091766	Crevice_feeder
tendybaguassu3	0.457088	0.242994	0.088302	Crevice_feeder
tendybaguassu4	0.460511	0.243543	0.088696	Crevice_feeder
tuca1	0.398263	0.223467	0.087711	Crevice_feeder
tuca2	0.433502	0.227815	0.090388	Crevice_feeder
tuca3	0.45425	0.236413	0.089363	Crevice_feeder
minuano1	0.400734	0.196683	0.094208	Molluscivore
minuano2	0.560056	0.250785	0.089959	Molluscivore
minuano3	0.490101	0.236918	0.09051	Molluscivore

minuano4	0.51457	0.223422	0.091049	Molluscivore
tesay1	0.497903	0.22504	0.097847	Molluscivore
tesay2	0.51895	0.216219	0.103386	Molluscivore
yaha1	0.453776	0.216435	0.090278	Molluscivore
yaha2	0.605408	0.26496	0.089163	Molluscivore
yaha3	0.60105	0.27998	0.086734	Molluscivore
yaha4	0.537904	0.248252	0.090996	Molluscivore
hadrostigma1	0.46105	0.215376	0.090623	Browser
hadrostigma3	0.514138	0.242843	0.08655	Browser
hadrostigma5	0.497652	0.247407	0.088579	Browser
tapii1	0.451773	0.217261	0.092156	Browser
tapii2	0.551511	0.257838	0.093841	Browser
tapii3	0.502236	0.236215	0.089661	Browser
tapii4	0.523824	0.242736	0.089331	Browser
tapii5	0.421713	0.207545	0.092936	Browser
tapii6	0.439976	0.213685	0.09068	Browser
tapii7	0.461521	0.22686	0.090527	Browser
aguaray_guazu1	0.565316	0.352994	0.075777	Piscivore
aguaray_guazu2	0.553048	0.4067	0.074769	Piscivore
aguaray_guazu3	0.513009	0.311727	0.080383	Piscivore
gillmorlisi1	0.48886	0.322608	0.074434	Macroinvertivore
gillmorlisi2	0.657803	0.389093	0.078705	Macroinvertivore

Footnote: These data were used to visually compare ecomorphs in a non-phylogenetic context (results shown in Figure 1c). For significance testing in a phylogenetic context see the data provided in Table S5 and Figure S5.

Table S5. Kinematic data (by population) used in the study.

Population/species	KT	Kinesis	KA	Prey_mobility
AguarayGuazu	0.543791	0.35714	0.076976	Evasive
gillmorlisi	0.573331	0.355851	0.076569	Semi_evasive
yjhui	0.486274	0.272976	0.084175	Evasive
tesay	0.508426	0.22063	0.100616	Non_evasive
tuca	0.428672	0.229232	0.089154	Non_evasive
iguassuensis	0.514786	0.317719	0.078454	Evasive
tapii	0.478936	0.228877	0.091305	Non_evasive
yaha	0.549534	0.252406	0.089293	Non_evasive
ypo	0.49605	0.298077	0.080319	Evasive
punctata	0.53245	0.321749	0.076611	Semi_evasive
maculata	0.644088	0.325498	0.083329	Semi_evasive
gaucho	0.616985	0.29592	0.085733	Semi_evasive
scottii	0.479379	0.319392	0.073823	Semi_evasive
iguapina	0.50715	0.292826	0.080119	Semi_evasive
missioneira_Toro	0.571767	0.327862	0.081833	Evasive
tendybaguassu_Toro	0.465181	0.236011	0.090473	Non_evasive
missioneira_Yaboti	0.541577	0.30991	0.081187	Evasive
tendybaguassu_Cuareim	0.458799	0.243268	0.088499	Non_evasive
hadrostigma	0.490947	0.235209	0.088584	Non_evasive
minuano_Yaboti	0.491365	0.226952	0.091431	Non_evasive

Footnote: These data match the tips of the SNAPP species tree and were used during the phylogenetic ANOVA and MuSSCRat analyses (results shown in Figures 1c, 2, and 3).

Table S6. Summary of gut content analyses.

species	n	fish	microinvertebrates	macrocrusaceans	mollusks	algae	classification_diet
celidochilus	30	91	9	0	0	0	Piscivore
missioneira	44	71	12	17	0	0	Piscivore
minuano	37	0	23	0	73	0	Molluscivore
tendybaguassu	26	0	90	0	7	3	Microinvertivore
hadrostigma	15	0	68	2	3	27	Omnivore
yjhui	5	81	19	0	0	0	Piscivore
iguassuensis	10	82	18	0	0	0	Piscivore
tesay	10	3	29	0	65	3	Molluscivore
tuca	5	9	77	2	8	4	Microinvertivore
tapii	10	4	57	0	5	34	Omnivore
scottii	19	24	29	43	4	0	Macroinvertivore
punctata	18	23	36	38	3	0	Macroinvertivore
vittata	27	100	0	0	0	0	Piscivore
hu	2	20	42	35	3	0	Macroinvertivore
ypo	16	65	23	12	0	0	Piscivore
mandelburgeri	5	61	33	6	0	0	Piscivore
taikyra	5	0	48	0	40	2	Molluscivore
yaha	5	0	29	0	68	3	Molluscivore
maculata	6	18	45	32	5	0	Macroinvertivore
iguapina	6	13	44	38	5	0	Macroinvertivore

Footnote: Data for *C. celidochilus*, *C. minuano*, *C. missioneira*, and *C. tendybaguassu* from Burress et al. (2013); data for *C. punctata*, *C. scottii*, and *C. vittata* from Burress et al. (2016); data for *C. hadrostigma* from Burress et al. (2018). All others from this study. Values are percent by volume.

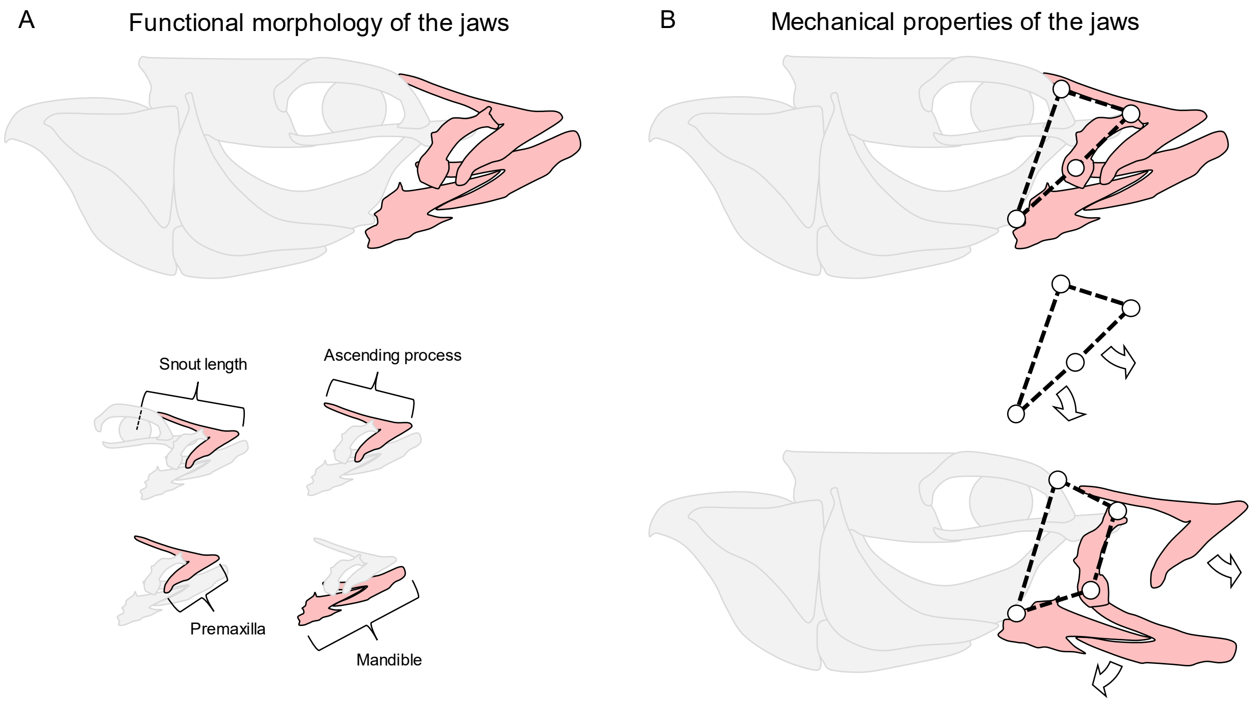

Figure S1. Traits measured to characterize the functional morphology of the oral jaws (a). Four-bar linkage system used to characterize the mechanical properties of the oral jaws (b).

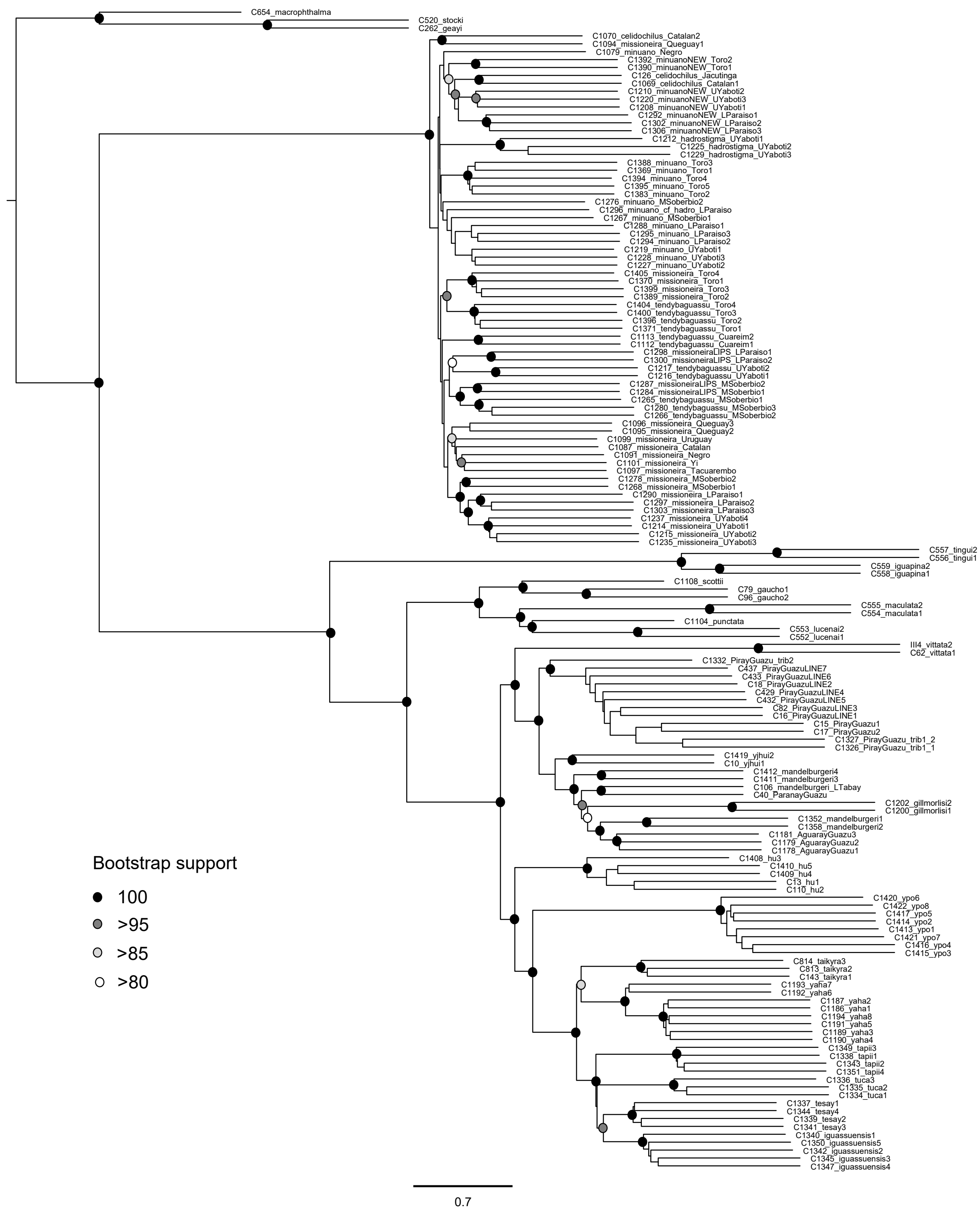

Figure S2. Full Astral-III species tree based on 147 individuals. Note that analyses in the paper were conducted on a reduced dataset including a single tip for every well-supported population (see Figure S3 and the main text for details). Support for nodes within populations are not depicted.

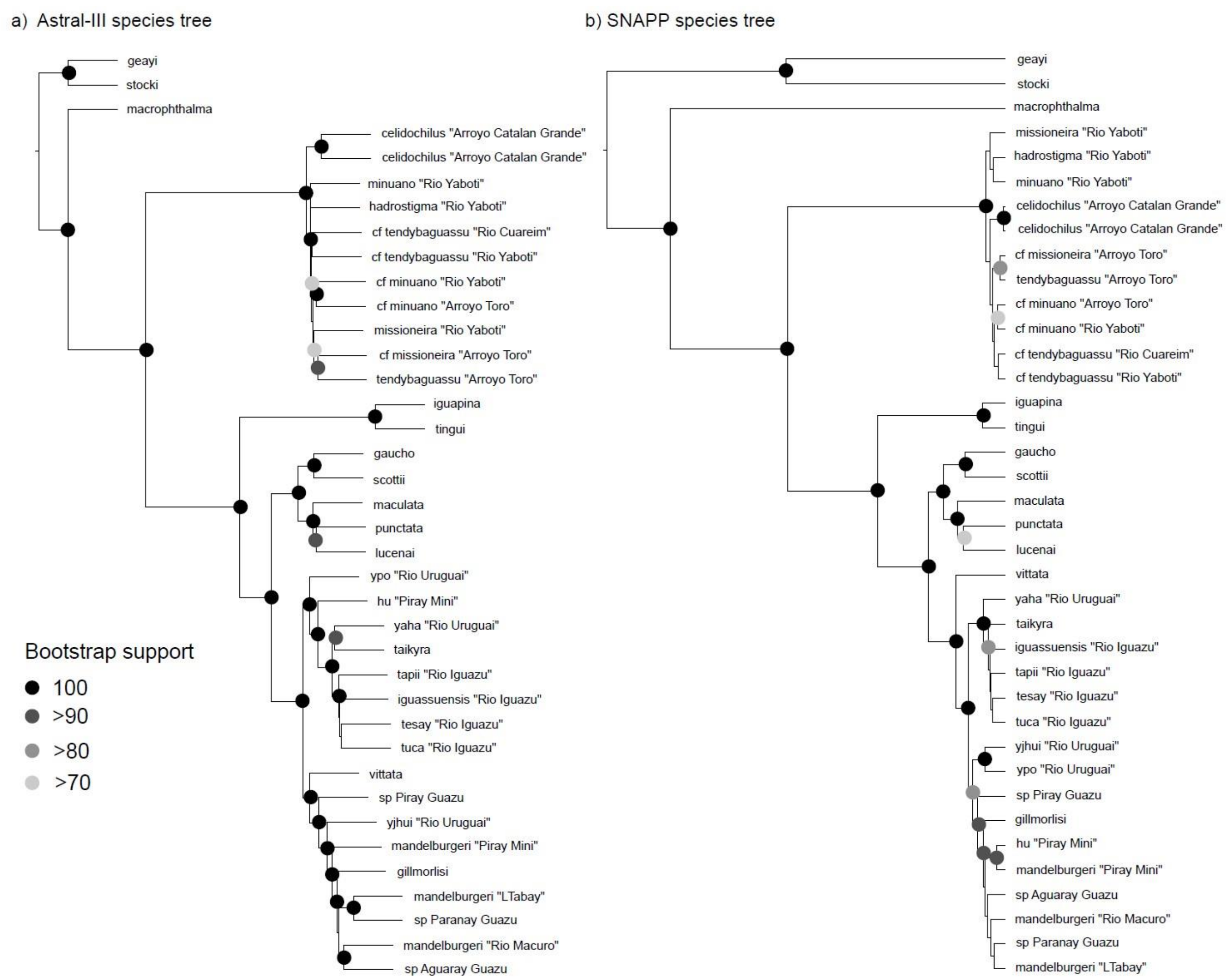

Figure S3. Phylogenomic hypotheses for pike cichlids of the La Plata River Basin based on a reduced sampling of 38 well-supported clades (reduced from Figure S1). a) ASTRAL-III and b) SNAPP tree. Note that the two *C. celidochilus* samples fall out together and were reduced to a single representative prior to subsequent analyses.

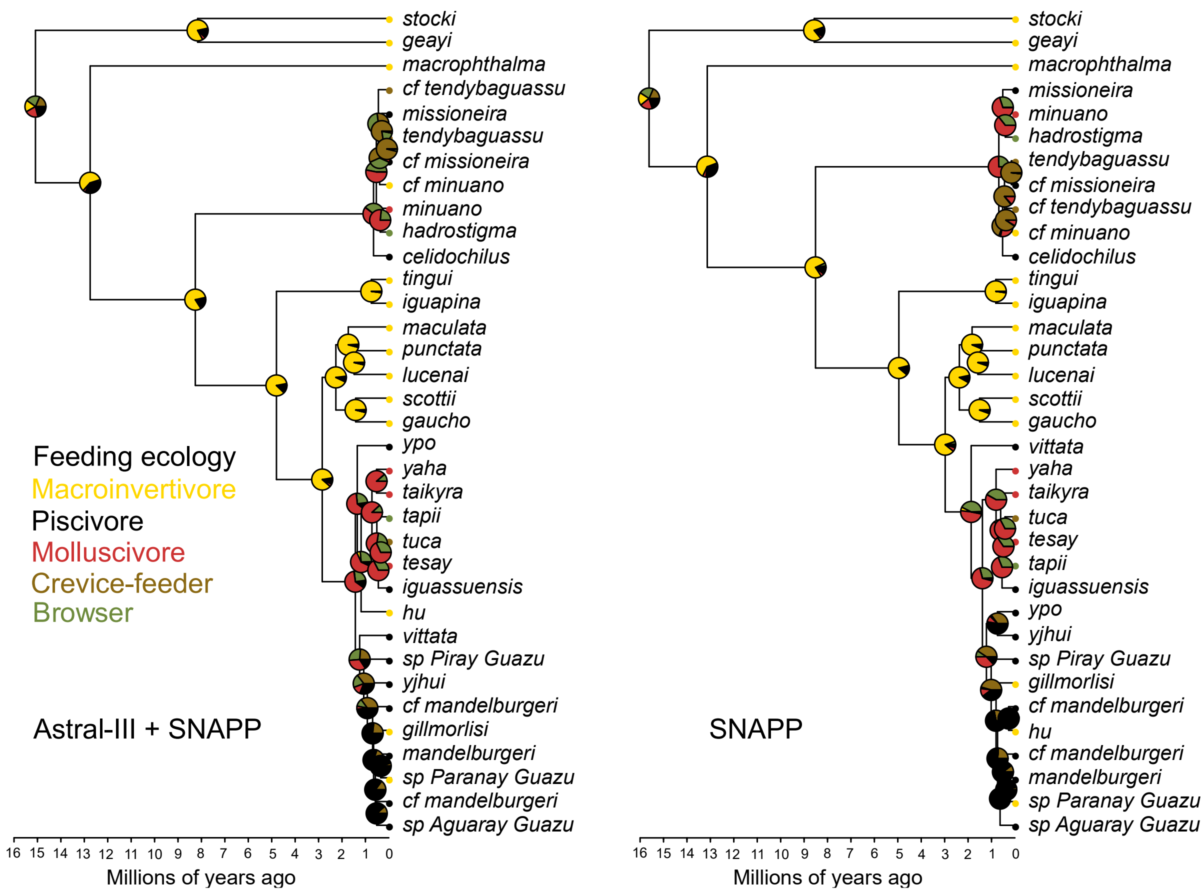

Figure S4. Evolutionary history of feeding ecology across a) SNAPP trees with a fixed Astral tree topology and b) SNAPP tree with no topological constraints.

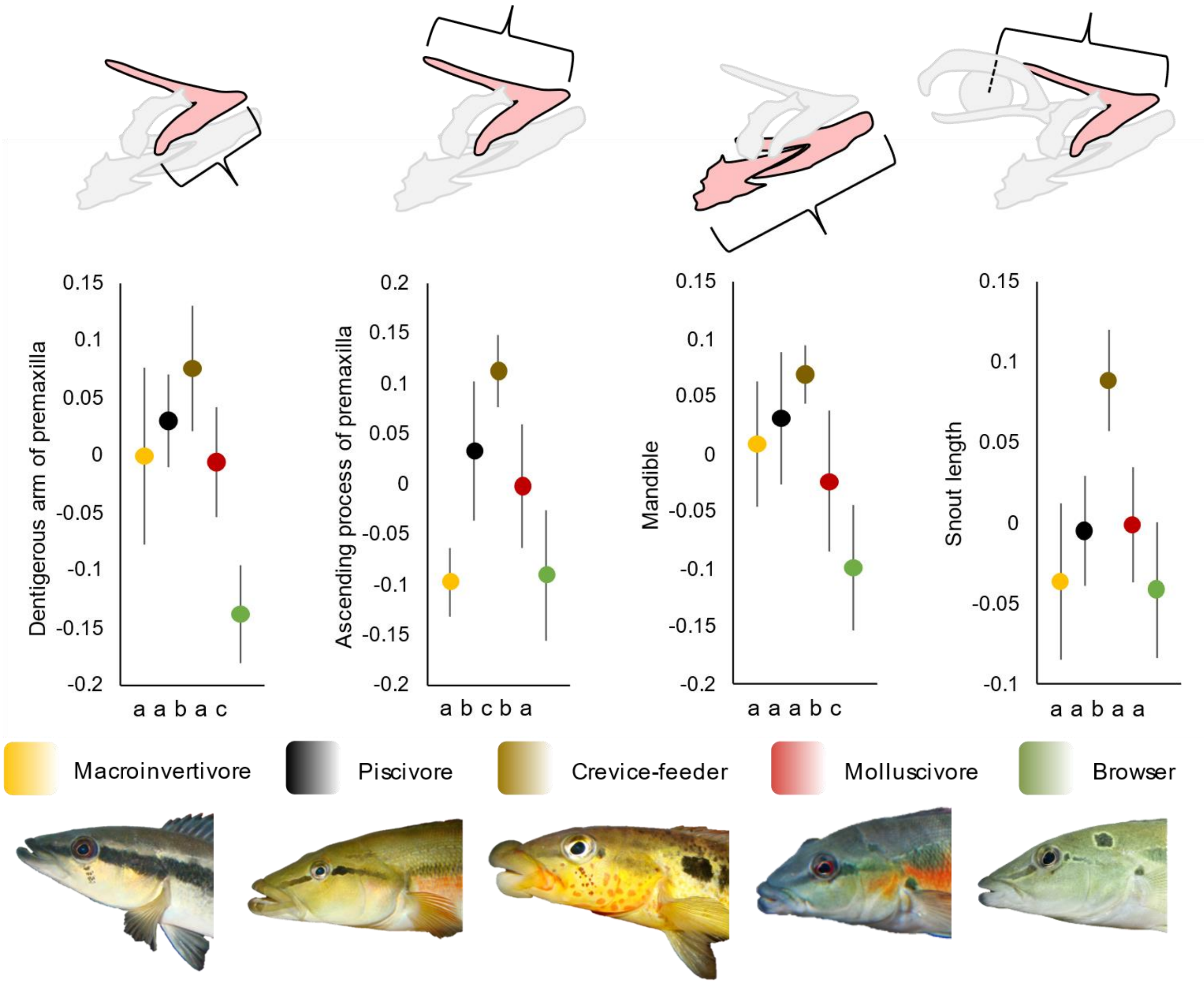

Figure S5. Morphological traits measured to characterize the shape of the oral jaws. Letters along the x-axis denote significant differences based on phylogenetic ANOVA. Illustrations depict the anatomical feature measured. Pictures depict a representative of each ecomorph (*Crenicichla scottii*, *C. celidochilus*, *C. tuca*, *C. minuano*, and *C. hadrostigma*; left to right). Photos of *C. minuano* and *C. hadrostigma* courtesy of Vin Kutty and Oliver Lucanus, respectively. All others by E.D.B.

ROBUSTNESS TO MODEL PRIORS

The posterior number of state changes were consistent across different priors on the number of rate shifts (13.1 to 16.4 state changes) (Figure S4). The posterior number of rates shifts varied based on the prior number of rate shifts, as expected (Moore et al. 2016; May and Moore 2020). Rates of morphological evolution were state-dependent across models with different priors on the number of rate shifts: 1 shift (PP = 1.0), 5 shifts (PP = 0.99), 10 shifts (PP = 0.98), and 15 shifts (PP = 0.914) (Figure S4).

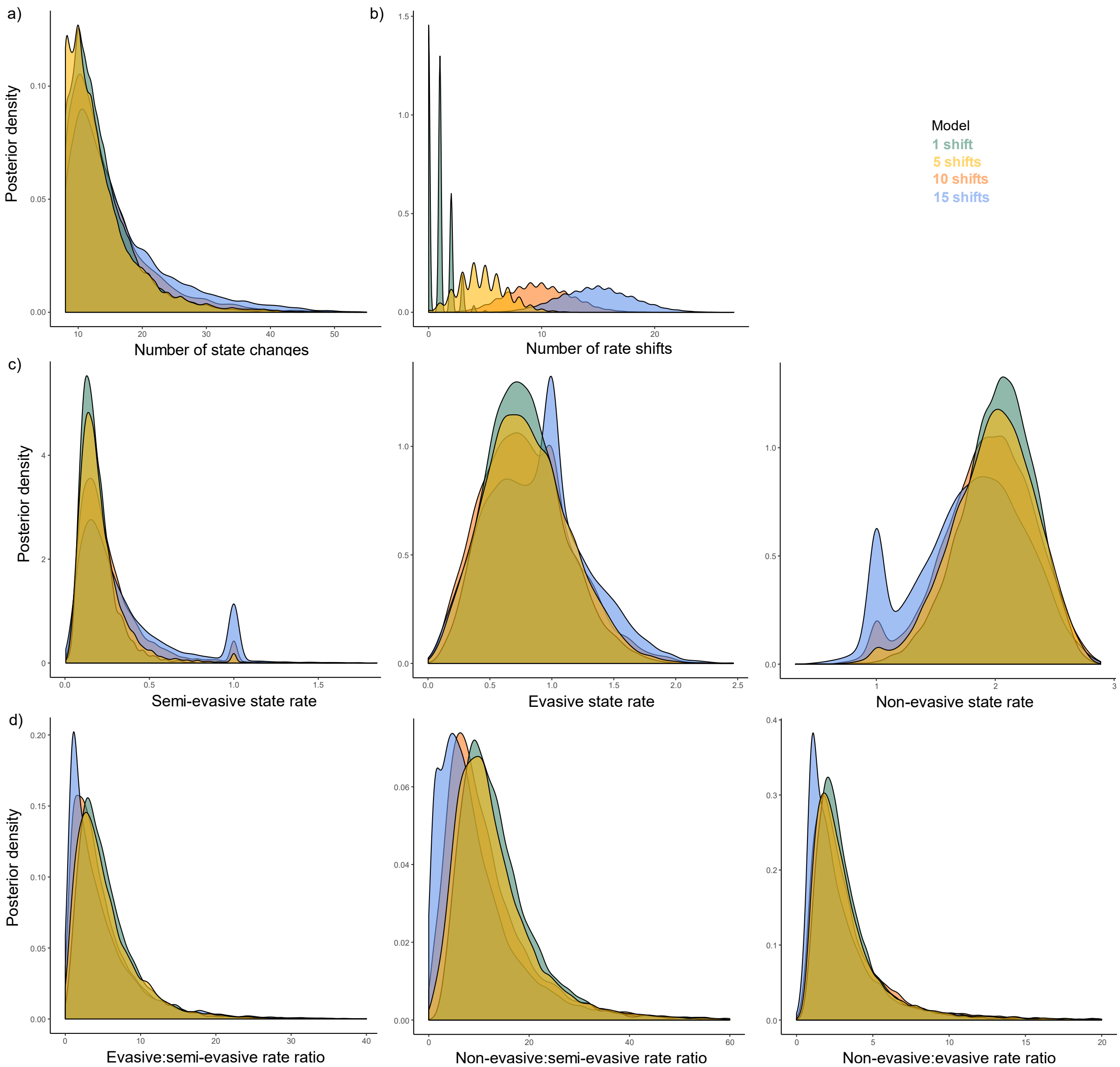

Figure S6. Posterior estimates of key parameters across models with different priors on the number of rate shifts: a) number of state changes, b) number of rate shifts, c) state rates, and d) rate ratios between state rates.

ROBUSTNESS TO MODEL OF EVOLUTION

In addition to the random local clock (RLC) model reported in the main text, we considered an uncorrelated lognormal (UCLN) model to estimate state-dependent rates of evolution (May and Moore 2020). The RLC model assumes a background rate at the root and subsequent branches draw a probability of a rate shift and either inherit the ancestral rate (in the absence of a shift) or draw a new rate. This model results in rates that have phylogenetic structure. In contrast, with the UCLN model each branch has its own background rate. Therefore, the rates lack phylogenetic structure. Based on the UCLN mode, rates of morphological evolution were state-dependent (posterior probability that rates were state-dependent; PP = 1.0), while rates of kinematic evolution were not (PP = 0.597) (Figure S5). This result was consistent with the results from the RLC model reported in the main text in which rates of morphological evolution were state-dependent (PP = 1.0), while rates of kinematic evolution were not (model averaged PP = 0.572). These results indicate that the state-dependent nature of evolutionary rates is not sensitive to the phylogenetic structure.

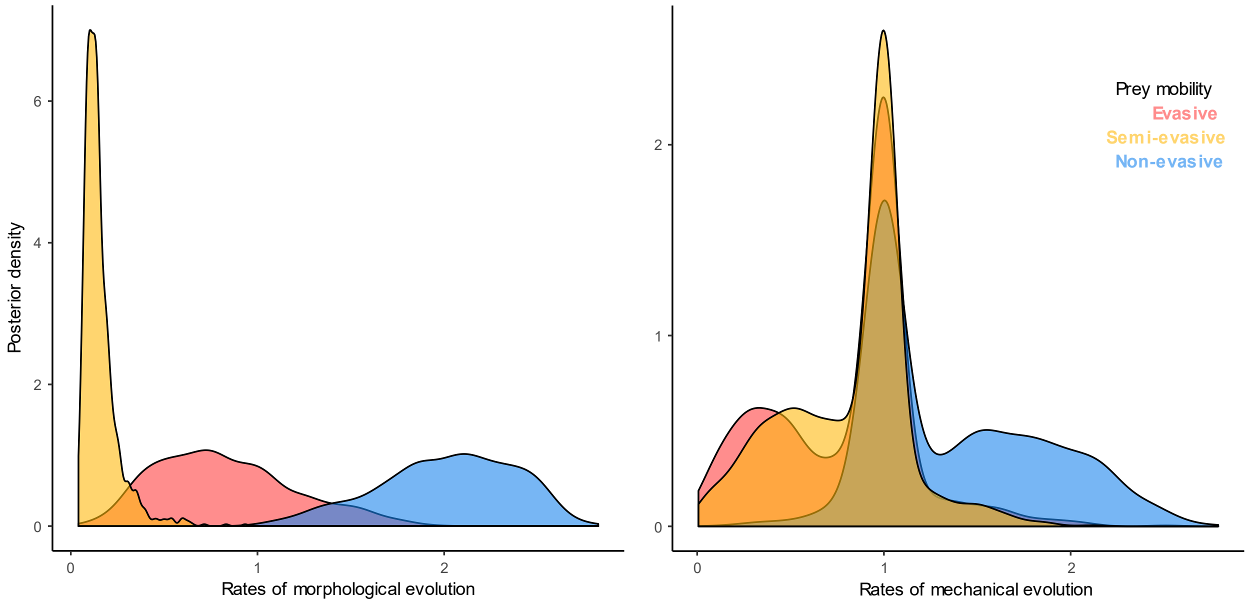

Figure S7. State-dependent rates of morphological (PP = 1.0) and mechanical evolution (PP = 0.597) based on an uncorrelated lognormal model (as opposed to the random local clock shown in the main text; depicted in Figure 2).

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