# Spotlight

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

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Abstract.--Explosive bouts of diversification are one of the most conspicuous features of the tree of life. When such bursts are repeated in similar environments, it suggests some degree of predictability in the evolutionary process. We assess parallel adaptive radiation of South American pike cichlids (*Crenicichla*) using phylogenomics and phylogenetic comparative methods. We find that species flocks in the Uruguay and Iguazú River basins rapidly diversified into the same set of ecomorphs that reflect feeding ecology. Both adaptive radiations involve expansion of functional morphology, resulting in unique jaw phenotypes. Yet, form and function were decoupled such that most ecomorphs share similar mechanical properties of the jaws (i.e., jaw motion during a feeding strike). Prey mobility explained 6- to 9-fold differences in the rate of morphological evolution but had no effect on the rate of mechanical evolution. We find no evidence of gene flow between species flocks or with surrounding coastal lineages that may explain their rapid diversification. When compared with cichlids of the East African Great Lakes and other prominent adaptive radiations, pike cichlids share many themes, including the rapid expansion of phenotypic diversity, specialization along the benthic-to-pelagic habitat and soft-to-hard prey axes, and the evolution of conspicuous functional innovations. Yet, decoupled evolution of form and function and the absence of hybridization as a catalyzing force are departures from patterns observed in other adaptive radiations. Many-to-one mapping of morphology to mechanical properties is a mechanism by which pike cichlids attain a diversity of feeding ecologies while avoiding exacerbating underlying mechanical trade-offs. [Adaptive radiation; ecological opportunity; feeding kinematics; functional trade-off; hybridization; introgression.]

Rapid bouts of in situ diversification are emblematic of many insular ecosystems such as islands and lakes (Seehausen 2006; Gavrilets and Losos 2009; Itescu 2019). This conspicuous pattern occurs in lineages widely distributed across the tree of life and globe, including Darwin's finches from the Galapágos islands, Hawaiian honeycreepers, silverswords, and stick spiders, Caribbean anole lizards, and East African cichlid fishes (Lack 1947; Carlquist 1974; Seehausen 2006; Grant and Grant et al. 2018; Losos 2009; Gillespie et al. 2018; Ronco et al. 2021). Islands and lakes are recognized as cradles of biodiversity (Seehausen 2006; Grant and Grant et al. 2018; Losos and Ricklefs 2009), and their proclivity for eliciting diversification is often attributed to release from competition and predation pressures (Schluter 1988) as well as access to new or underutilized resources (Simpson 1953; Stroud and Losos 2016).

From a theoretical perspective, many sources of ecological opportunity should apply similarly to other types of geographic transitions (i.e., other than islands and lakes). For example, abrupt invasion of a new river basin via stream capture (Bishop 1995) or a new landmass via dispersal across a land bridge (Noonan and Chippindale 2016; Říčan et al. 2013) should provide ecological opportunity similar to invading an island from the nearby mainland or a lake from surrounding rivers. Despite that most theory has been based on islands (Gavrilets and Losos 2009; Stroud and Losos 2016), colonizing new environments, including lakes and rivers, appear to elicit bouts of speciation in cichlids (Burress and Tan 2017) and numerous "continental radiations" exhibit general patterns consistent with ecological opportunity and adaptive radiation (Derryberry et al. 2011; Arbour and López-Fernández 2016; García-Navas et al. 2018). Yet, there are discrepancies in the biogeographic circumstances in which adaptive radiation unfolds.

Cichlid fishes are widespread throughout the Neotropics and Africa, but a large part of their diversity is concentrated into a handful of lakes in East Africa (Seehausen 2015). Lakes Tanganyika, Malawi, and Victoria house their own species-rich and ecologically diverse cichlid assemblages, including considerable phenotypic convergence within (Muschick et al. 2012) and among lakes (Kocher et al. 1993). There has been extensive in situ phenotypic evolution in the East African Great Lakes (Young et al. 2009; Ronco et al. 2021) as well as numerous smaller lakes throughout Africa and Middle America (Elmer et al. 2014; Martin et al. 2015; Ford et al. 2016). Similar evolutionary patterns have been conspicuously lacking in rivers (Burress 2015; Seehausen 2015). In contrast to lakes, rivers are more continuous and labile environments. Heterogeneous and unpredictable environmental conditions (e.g., flow regimes, turbidity,

temperature, resource availability, etc.) may weaken the selection necessary to drive specialization, instead favoring opportunistic ecological roles such as omnivory (Winemiller 1990; Jepsen and Winemiller 2002). Heavy predation pressure from older and saturated riverine communities may also constrain diversification (Schluter 1988; McGee et al. 2020). Rivers also frequently have a constrained spatial dimension (i.e., depth; with notable exceptions such as the vastly deep Congo River), which has been a crucial environmental axis along which diversification has ubiquitously occurred in lakes (Cooper et al. 2010; Hulsey et al. 2013). In particular, depth produces gradients in light, oxygen, and temperature that may be exploited during adaptive radiation. These are possible factors that may explain the relatively lower degree of rapid ecological divergence in many riverine species flocks (e.g., Schwarzer et al. 2011; Alter et al. 2017).

Here, we evaluate the diversification of pike cichlids (Crenicichla) within the La Plata River Basin, with particular focus on two species flocks that appear to share some features with lacustrine cichlid adaptive radiations (Piálek et al. 2012; Burress et al. 2018a,b; inset in Fig. 1). Most of their ecological diversity is distributed in two groups-one in the Paraná River basin within the Iguazú River and the other in the Uruguay River basin (Piálek et al. 2012; Burress et al. 2018b; Piálek et al. 2019). In each river basin, the resident assemblage consists of a clade of endemic species that co-occur throughout the basin (in the Uruguay, Middle Paraná, and Iguazú Rivers; Piálek et al. 2012, 2015; Serra et al. 2016). These clades have evolved into similar sets of ecomorphs defined by the shape of their heads and pharyngeal jaws, which closely reflects their feeding ecology (Burress et al. 2018b; inset in Fig. 1).

In this study, we scrutinize the Crenicichla species flocks in light of emergent patterns from cichlid adaptive radiations in lake ecosystems. First, lake assemblages tend to exhibit in situ, and often rapid, ecological, and phenotypic evolution (Hulsey et al. 2010; Elmer et al. 2014). Second, functional morphology and mechanical properties of the jaws (i.e., motion during a feeding strike) are strongly evolutionarily coupled (Martinez et al. 2018). Third, inherent attributes of prey, such as their evasive nature, underlies much of the functional and mechanical diversity of the jaw system, in particular, extensive specialization along the velocity-force mechanical trade-off (Albertson et al. 2003; Cooper et al. 2010; McGee et al. 2016; Martinez et al. 2018). Lastly, hybridization often functions as a catalyst of adaptive radiation, including in Lake Tanganyika (Irisarri et al. 2018), Malawi (Svardal et al. 2020), Victoria (Meier et al. 2017), and many smaller lakes throughout Africa (Martin et al. 2015; Meier et al. 2019) and Middle America (Kautt et al. 2016, 2020). To evaluate if these four patterns are present in Crenicichla, we (i) provide a time-calibrated phylogenomic hypothesis for the group, (ii) estimate the evolutionary history of feeding ecology, (iii) evaluate the evolution of functional and mechanical properties

of the jaws in response to feeding ecology and prey evasiveness, and (iv) estimate gene flow across the radiation. We then place this riverine lineage alongside the well-established adaptive radiations in the literature and discuss commonalities and points of conflict.

#### MATERIALS AND METHODS

#### Taxon Sampling

Pike cichlids were sampled across their range in subtropical South America, including Argentina, Brazil, and Uruguay (Table S1 of the Supplementary material available on Dryad at https://doi.org/10.25338/B8XP7D): a total of 147 individuals representing 37 species/populations, including all known ecomorphs (Table S1 of the Supplementary material available on Dryad).

We selected three additional outgroup species based on published studies of the molecular phylogeny of pike cichlids (Piálek et al. 2012, 2019; Burress et al. 2018b). Fishes were collected in accordance with Ministerio de Ecologia y Recursos Renovables, Misiones, Argentina (Resolucion 509/07 and 071), Administración de Parques Nacionales, Argentina (NEA 224, 328 and 35), and La Dirección Nacional de Recursos Acuáticos, Montevideo, Uruguay (Resolucion 202/1383/2010 and 13/2014).

## Restriction Site-Associated DNA-Tags Processing

For details about the restriction site-associated DNA (RAD) library preparation, see Supplementary materials available on Dryad. Raw reads were filtered, aligned and variants called in the Stacks package (v2.4; Catchen et al. 2011; Rochette et al. 2019). Barcode sorting and quality filtering of raw reads were performed in process\_radtags program (Stacks; with default setting). Sequencing of 147 samples representing 37 potential species resulted in 442.9 million sequenced fragments of which 428.9 million (96.8%) passed through the procedure of barcode sorting and quality filtering. One sample was represented in average by 2,917,668  $\pm$  1,099,661 SE retained reads. The filtered reads were aligned into homologous loci based on mapping onto a reference genome; we used a draft genome of Crenicichla semifasciata obtained from deep Illumina sequencing of a 10× Genomics library (assembly size = 735 Mb, scaffold N<sub>50</sub> = 11.1 Mb). Mapping was performed using Bowtie 2 assembler (v2.2.4; Langmead and Salzberg 2012; "-very-sensitivelocal" preset mode of parameters) and then processed in the ref\_map pipeline (Stacks). Single nucleotide polymorphism (SNP) variants calling was processed in the population program (Stacks). Alignments of all samples (in the form of BAM files), including sequences of the reference genome were deposited into the Gen-Bank Sequence Read Archive (project No. PRJNA705376; http://www.ncbi.nlm.nih.gov/sra/PRJNA705376).

## Phylogenetic Inference and Divergence Time Estimation

To infer a species tree, we used Accurate Species TRee ALgorithm (ASTRAL), a genome-scale coalescent-based

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FIGURE 1. Parallel diversification of pike cichlids in the Uruguay and Iguazú Rivers. a) Ancestral reconstruction of feeding ecology across the SNAPP species tree. b) Functional morphology associated with feeding ecology (i.e., ecomorphs). Points depict individuals. c) Mechanical properties of the jaws among ecomorphs (mean  $\pm$  95% confidence interval). Inset illustrations and pharyngeal jaws (top-to-bottom): *Crenicichla missioenira*, *C. minuano*, *Crenicichla hadrostigma*, *Crenicichla celidochilus*, *C. tendybaguassu*, *C. iguassuensis*, *Crenicichla tapii*, *C. tuca*, *C. tesay*, and *C. yjhui*.

species tree estimation from gene trees introduced by Mirarab et al. (2014). Complete sequences of variable loci were exported from Stacks (in diploid FASTA format: "-fasta-samples" option in populations; loci with >90% of samples present exported). A strict consensus from both allele sequences of each sample was made in FASconCAT-G (Kück and Longo 2014) and exported in PHYLIP format. Because the number of SNPs within a single RAD locus (which length was 147.2 bp in average) is too low to infer a phylogenetic tree (an average 6.6 variant sites including parsimonyuninformative ones per locus), several nearest variable loci along the reference genome were grouped together into one "superlocus." To find the optimal aggregation mode (providing a well-resolved ASTRAL tree although keeping the number of aggregated loci as low as possible to avoid grouping of distant loci) we inferred ASTRAL trees based on aggregated sets of the nearest 5, 10, 15, 20, and 25 loci for comparison. First, maximum-likelihood trees were inferred for each of the "superloci" in all aggregation modes (3667, 1833, 1222, 916, and 733 trees, respectively) in RAxML (v8.2.4; Stamatakis 2014). The final ASTRAL supertree was then constructed from them in the ASTRAL-III (Zhang et al. 2018) program involving an improved method of the ASTRAL algorithm. For each of the resulting ASTRAL supertrees, we counted a sum of node supports and choose the optimal aggregation mode to be 10 loci based on the supertree with maximum summed support (124.42, 127.88, 127.32, 124.33, and 124.45, respectively).

To estimate the divergence times of the ASTRAL tree, we used SNAPP (v1.4.0; Bryant et al. 2012), an add-on package for the BEAST program (v2.5.0; Drummond and Rambaut 2007), implementing a method for phylogenetic inference with the multispecies-coalescent model based on SNP data. To overcome some limitations of this method (Matschiner et al. 2017; Stange et al. 2018), we follow the approach described in Stange et al. (2018) for XML input file preparation (using their snapp\_prep.rb script); the computations were run with 1000 randomly filtered SNPs. To estimate divergence times, we ran two SNAPP iterations: the first with a fixed topology throughout the SNAPP analysis (by using the Astral-III topology as a starting tree and excluding the treeNodeSwapper operator) and the second without any topology constraints. Markov Chain Monte Carlo (MCMC) sampler convergence was determined based on the run statistics provided in Tracer v1.7.1 (Rambaut et al. 2018) considering the effective sample size value of all parameters greater than 200 as the main criterion (1,000,000 of generations for the fixed topology; 3,000,000 without constraints). For all SNAPP trees calibration, we constrained the crown divergence of the clade comprising the Crenicichla reticulata species group (Crenicichla stocki, Crenicichla geayi), Crenicichla macrophthalma and the Crenicichla lacustris species group. The age of this node was estimated at 16.77 Ma (95% Highest Posteior Density (HPD) interval 10.48-22.61) and was input as a lognormal distribution centered at 16.77 Ma with SD =0.229. This date is derived from a calibration of the basal node of the genus *Crenicichla* estimated by Musilová et al. (2015) at 28 Ma (SD =1). The dated phylogeny of Musilová et al. (2015) falls well within the bounds established by the review of Neotropical cichlid dating studies (Pérez-Miranda et al. 2020).

## Functional Morphology of the Jaws

To characterize functional feeding morphology, we measured four linear measurements related to the shape, size, and protrusion of the oral jaws from 128 individuals representing 24 species (Tables S2 and S3 of the Supplementary material available on Dryad). These species were chosen to represent the ecological and morphological diversity of the C. lacustris species group (Lucena and Kullander 1992; Burress et al. 2013; Piálek et al. 2015; Burress et al. 2018a,b). Measurements included the length of the lower jaw, measured from the anterior tip of the mandible to the articular-quadrate joint; the length of the dentigerous arm of the premaxilla (upper jaw), measured as the linear distance from the anterior midline to the tip of the dentigerous arm; the ascending process of the premaxilla, measured as the linear distance from the base of the dentition at the midline to the dorsal tip of the ascending process; and snout length, measured as the linear distance from the center of the eye to the anterior tip of the upper jaw (illustrated in Fig. S1a of the Supplementary material available on Dryad). These measurements are correlated with gape, protrusion, prey size, and mechanical properties of the feeding motion in fishes (Mittelbach and Persson 1998; Hulsey and De León 2005; Hulsey et al. 2010). Each trait scaled strongly with body size and was transformed to relative distances using residuals derived from regressing ln-transformed trait values against lntransformed standard body length (i.e., the distance from the tip of the upper jaw to the base of the caudal fin). Species/population means were used in subsequent analyses.

## Mechanical Properties of the Jaws

To describe mechanical properties that characterize the feeding motion of the jaws, we measured kinematic transmission (KT; Westneat 1990), kinesis, and kinematic asynchrony (KA) from 61 individuals representing 20 species (Tables S4 and S5 of the Supplementary material available on Dryad). These traits are properties of the oral jaw four-bar linkage system (illustrated in Fig. S1b of the Supplementary material available on Dryad) that we calculated based on the methods by Martinez and Wainwright (2019). KT represents the ratio of output rotation of the four-bar's maxillary link to the input rotation of the mandible. Generally speaking, larger values indicate a jaw that transfers more velocity, whereas smaller KT values indicate a jaw that transmits more force. Kinesis is a measure of four-bar mobility. Generally speaking, larger values reflect more motion of the four-bar shape. KA reflects the relative degree of temporal asynchrony of mobile

components during the feeding motion, calculated as the maximum deviation from linear (i.e., a straight line from the starting to ending four-bar shapes, after rotation). Generally speaking, larger values indicate a less mechanically efficient feeding motion than smaller values. To determine an appropriate starting angle for these calculations, we measured the angle between the fixed and lower jaw links while the mouth was closed. This angle varied between 30° and 46° among species. To ensure comparability among species, we used the mean angle of 39° for calculating KT, kinesis, and KA. Species/population means were used in subsequent analyses.

## Phylogenetic Comparative Methods

We classified species into five guilds based on assessments of their feeding ecology: piscivores, macroinvertivores, molluscivores, crevice-feeders, and browsers (Table S2 of the Supplementary material available on Dryad). These classifications were based on two complementary types of data: (i) analyses of gut contents and (ii) detailed assessments of pharyngeal jaw dentition. Presence and diversity of tooth types on the upper and lower pharyngeal jaw (following Casciotta and Arratia 1993) are known to strongly reflect the dietary patterns in cichlids (Burress 2016), including Crenicichla specifically (Burress et al. 2013; Kuhn et al. 2020; See Table S2 and the Supplementary material available on Dryad for a detailed account of the classification system). Briefly, piscivores eat almost exclusively fishes and possess largely simple and recurved conical pharyngeal teeth (1–2 tooth types). Macroinvertivores 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 beveled 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 rockclinging 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. We then estimated the evolutionary history of these feeding ecologies using 1000 stochastic histories implemented in phytools (Revell 2012). During this procedure, we used a transition model that permitted transition rates to vary (i.e., an all-rates-different model [ARD]), which was preferred over a model with fixed transition rates (i.e., an equal-rates model [ER]) based on Akaike Information Criterion.

We compared functional morphology and feeding kinematics among the ecomorphs using phylogenetic Analysis of Variance (ANOVA) with a residual randomization permutation procedure (Collyer and Adams 2018) implemented in Geomorph (Adams and Otárola-Castillo 2013). Statistical significance was determined using 10k permutations. Further, we determined the statistical significance of pairwise comparisons using the pairwise function implemented in the RRPP package (Collyer and Adams 2018).

We further classified the species in terms of prey mobility (Table S2 of the Supplementary material available on Dryad). Evasive prey includes organisms capable of sustained and rapid evasion (e.g., fishes). Semievasive prey includes organisms with limited ability to evade predators, either in the duration or speed of movement (e.g., shrimps and crabs). Nonevasive prey includes organisms that lack the ability to evade predators (e.g., attached prey such as periphyton and snails) or otherwise have a very limited capacity to do so (e.g., rock-clinging insects).

We tested the effect of prey mobility, a discrete character, on the rates of morphological and mechanical evolution using multiple state-specific rates of continuous-character evolution (MuSSCRat; May and Moore 2020) implemented in RevBayes (Höhna et al. 2016). This model incorporates background rate variation (i.e., rate variation not attributed to the discrete character), thereby reducing the risk of erroneously attributing rate heterogeneity to the discrete character of interest. This model also accommodates multivariate continuous characters and simultaneously estimates the evolutionary history of the discrete and continuous characters (rather than estimating each sequentially). The MCMC models were run for 10k generations with 10% burnin. We used a random local clock (RLC) model to estimate the state-dependent rates. MuSSCRat requires a prior on the number of rate shifts for the continuous characters. Therefore, we repeated analyses with different priors (1, 5, 10, and 15 shifts) to assess its impact on posterior estimates of key parameters (i.e., the number of state changes, the number of rate shifts, and estimates of state-dependent evolutionary rates). To further scrutinize the robustness of results, we repeated the MuSSCRat analyses using an uncorrelated log-normal model (UCLN; May and Moore 2020). The RLC and UCLN models differ principally in that the evolutionary rates have phylogenetic structure versus no phylogenetic structure, respectively.

#### Testing for Gene Flow Involving the Species Flocks

To test for potential gene flow, we computed Patterson's D (ABBA-BABA) and related statistics using Dsuite v0.4 r41 (Malinsky et al. 2021). This package allows calculations of the D and  $f_4$ -ratio statistics across all combinations of populations or species directly from a variant call format file (obtained from RAD-tags processing in stacks). Because these metrics involve fitting a simple explicit phylogenetic tree model to a quartet of species, it provides a formal test of admixture



FIGURE 2. Morphological and mechanical diversification of the jaws in response to prey mobility. a) Rates of morphological evolution as a function of prey mobility (PP = 1.0). b) Rates of mechanical evolution as a function of prey mobility (PP = 0.56). Inset images depict prey items that characterize each category: evasive (fishes; *Astyanax* spp. and *Gymnogeophagus labiatus*), semievasive (macrocrustaceans; *Trichodactylus panoplus*, *Aegla uruguayana*, and *Parastacus* sp.), and nonevasive (snails, rock-clinging insect larvae, and algae). Illustrations depict jaw morphologies associated with the extremes of prey mobility. Photos by E.D.B.

in a historical context. The system of  $f_4$ -ratio results were interpreted with the use of the *f*-branch metric (Malinsky et al. 2018) assigning gene flow evidence to specific branches of the phylogeny (computed also in Dsuite with the Fbranch program and associated plotting utilities).

We calculated the *f*-branch statistic for all relevant combinations of species/populations and branches, but focus on allele sharing between the Iguazú and Uruguay River species flocks and between a species flock and adjacent Atlantic coastal drainages in Brazil and Uruguay. These lineages lack contemporary, but have historically had, geographic connections, evidenced by the distributions of many fishes that span the Paraná River, Uruguay River, and Atlantic coastal drainages (Volcan et al. 2012; Delariva et al. 2019; Smith et al. 2021). These comparisons best approximate the geographic scenario of gene flow between riverine lineages and endemic lacustrine species flocks (Joyce et al. 2011; Meier et al. 2017, 2019).

## RESULTS

The Astral and SNAPP species trees were wellresolved and supported (Figs. S2 and S3 of the Supplementary material available on Dryad). Topologies differed most notably in the positions of *Crenicichla vittata*, *Crenicichla hu*, *Crenicichla ypo*, and within the *Crenicichla missioneira* complex but otherwise the topologies were highly congruent (Fig. S3 of the Supplementary material available on Dryad). The macroinvertivore guild was the ancestral state throughout the deeper nodes of the phylogeny (Fig. 1a and Fig. S4 of the Supplementary material available on Dryad). Most of the ecological diversity was concentrated into the Uruguay River and the Iguazú River species flocks (Fig. 1a). The ecological diversity of both these species flocks most likely arose from a molluscivorous ancestor (Fig. 1a). Feeding guilds were easily distinguished by oral jaw functional morphology (Fig. 1b and Fig S5 of the Supplementary material available on Dryad), pointing to discrete ecomorphs. KT did not vary among ecomorphs, but the crevice-feeder, molluscivore, and browser ecomorphs had less oral jaw kinesis and less mechanically efficient feeding motions than the piscivore and macroinvertivore ecomorphs (Fig. 1c).

Rate of morphological evolution responded strongly to prey mobility (posterior probability [PP] = 1.0). Evasive prey elicited 3- to 4-fold faster morphological evolution than semievasive prey, whereas nonevasive prey elicited 5- to 11-fold faster rates (PP = 0.91-1.0; Figs. 2 and 3a). In contrast, there was no difference in rate of mechanical evolution (PP < 0.655; Fig. 2). Background rate variation, rate heterogeneity not attributed to prev mobility, was distributed differently for morphological and mechanical traits (Fig. 3b and c). For morphological traits, background rate variation included elevated rates in the Uruguay River and Iguazú River species flocks (Fig. 3b), whereas elevated background rates of mechanical evolution were largely isolated to the crevice-feeder ecomorphs and their respective sister species in both species flocks (Fig. 3c). Background rate variation of morphological and mechanical evolution included slower rates associated with the Crenicichla *yjhui* and *C. ypo* species pair (Fig. 3b and c). There was also a discrepancy in the magnitude of background



FIGURE 3. Rates of morphological and mechanical evolution among pike cichlids across the SNAPP species tree. a) State-dependent and b) background rates of morphological evolution and c) background rates of mechanical evolution. Background rates are heterogeneity not attributed to the discrete character (i.e., prey mobility). Note that rates of mechanical evolution were not state-dependent, so background rate variation is representative of the overall rate heterogeneity.

rate variation. There was 1.6- and 3.2-fold variation in background rates of morphological evolution and mechanical evolution, respectively, likely reflecting that prey mobility explained a large fraction of variation in rates of morphological but not mechanical evolution. Results were consistent across runs with different priors (Fig. S6 of the Supplementary material available on Dryad) and between RLC and UCLN models (Fig. S7 of the Supplementary material available on Dryad).

The *f*-branch statistic indicates there are no genetic polymorphisms shared between the Uruguay and Iguazú River species flocks or between the Atlantic coast lineages and the Uruguay River flock (Fig. 4). We found very few shared polymorphisms between the coastal lineages and the Iguazú River (Fig. 4). Based on the low numbers of ABBA and BABA patterns and small imbalance between them, we suggest this is evidence for more ancestral rather than contemporary or recent gene flow (e.g., *Crenicichla maculata* and *Crenicichla punctata* share derived alleles with all four species from the Iguazú River; Fig. 4). Because these instances are not strong and limited to a few taxa, we conclude

that it is very unlikely that allele sharing between major drainages, including between the Uruguay and Iguazú River species flocks or between the species flock and Atlantic coast lineages (Fig. 4), contributed to the patterns of adaptive radiation exhibited by both species flocks. There is more extensive gene flow between smaller drainages within the Paraná River (e.g., *C. yjhui* and *Crenicichla mandelburgeri*), within the Iguazú River (e.g., *Crenicichla iguassuensis* and *Crenicichla tesay*), between the Iguazú River and Paraná River (e.g., *C. tesay, Crenicichla yaha*, and *Crenicichla taikyra*), and within the Uruguay River (Fig. 4). Therefore, gene flow may partially explain the species whose positions are discordant between the ASTRAL and SNAPP trees.

#### DISCUSSION

## *Emergent Themes at the Intersection of Macroevolution and Adaptive Radiation*

Pike cichlids exhibit parallel adaptive radiation emblematic of other groups such as Caribbean anoles,



FIGURE 4. Degree of allele sharing between species/populations and ancestral branches. The values (i.e., shading) in the matrix depict the excess allele sharing the branch identified along the *y*-axis (relative to its sister branch) and the species identified along the *x*-axis. Regions of the matrix that correspond to allele sharing between species flocks (solid lines) and between species flocks and Atlantic coast lineages (dashed lines) are highlighted. Note that *Crenicichla scottii* occurs in the Uruguay River as well as numerous smaller Atlantic coast drainages.

East African cichlids, and Hawaiian stick spiders (Kocher et al. 1993; Losos 2009; Gillespie et al. 2018). The small-scale, endemic, ecologically diverse radiations observed in the Uruguay and Iguazú Rivers are reminiscent of the species flocks of cichlids in a small crater, volcanic, and soda lakes (Schliewen et al. 2001; Elmer et al. 2014; Martin et al. 2015; Ford et al. 2016), as well as African barbs in volcanic lakes and rivers (Nagelkerke et al. 1994; Levin et al. 2020), Caribbean pupfishes (Martin et al. 2017), and Sulawesi sailfin silversides (Herder et al. 2006; Pfaender et al. 2011). There are several environmental dimensions that are commonly exploited by these groups, including the benthic-pelagic habitat axis and soft-to-hard prey axis (Seehausen and Wagner 2014). The *Crenicichla* species flocks involve a mixture of pelagic predators (Burress et al. 2013; Piálek et al. 2019) and an assortment of benthic-oriented specialists (Fig. 1). The benthic-pelagic habitat axis is nearly ubiquitously used during cichlid adaptive radiations in lakes (Cooper et al. 2010; Hulsey et al. 2013), as well as at broader macroevolutionary scales in fishes (Ribeiro et al. 2018; Friedman et al. 2020). Similarly, in the terrestrial realm, the ground-arboreal habitat dimension has also been liberally used during adaptive radiation (e.g., anoles; Losos 2009). The soft-to-hard prey axis is also a major environmental axis exploited by the *Crenicichla* species flocks (Fig. 1), mirroring ecological divergence patterns of cichlids in African and Nicaraguan lakes (Kidd et al. 2006; Muschick et al. 2011; Elmer et al. 2014), as well as broader macroevolutionary scales (Hulsey et al. 2008; Burress 2016).

There are, however, some differences between the pike cichlid adaptive radiations and some of the aforementioned groups that inhabit lakes. For example, in Tanganyikan and Malawian cichlids, functional morphology and mechanical properties of the jaws are strongly evolutionarily coupled (Martinez et al. 2018), whereas these features are largely decoupled in pike cichlids (Figs. 2 and 3). It is unclear if these are lineagespecific outcomes or are more broadly attributable to ecosystem-specific outcomes driven by inherent differences between rivers and lakes. Decoupling of functional morphology and mechanical properties of the jaws in response to feeding ecology is also apparent over the long evolutionary history of marine fishes (Corn et al. 2020). Similarly, the extremes of feeding ecology elicit elevated rates of phenotypic evolution in marine reef fishes (Borstein et al. 2019). Both these phenomenadecoupling of form and function and a diversifying effect of functional extremes—are apparent in pike cichlids and arose over short time scales (<1 My; Figs. 1 and 2). Our study highlights the tremendous range of time scales at which these macroevolutionary patterns may emerge.

## Hybridization as a Catalyst of Adaptive Radiation

There is mounting evidence that hybridization plays a critical role in triggering adaptive radiation (Salzburger 2018; Svardal et al. 2021), including in the East African Great Lakes (Joyce et al. 2011; Meier et al. 2017; Irisarri et al. 2018; Svardal et al. 2020), as well as smaller lakes throughout Africa (Martin et al. 2015; Meier et al. 2019) and Middle America (Kautt et al. 2016). Hybridization contributes to structural variation and genetic diversity and, in particular, gene duplications and transposable elements may play key roles during adaptive radiation (Svardal et al. 2021). An emergent pattern in the East African Great Lakes is the occurrence of hybridization with surrounding riverine lineages (Joyce et al. 2011; Meier et al. 2017, 2019). An analogous scenario in pike cichlids would be hybridization between a species flock and adjacent Atlantic coast lineages in Brazil and Uruguay with which they are closely related but lack contemporary geographic access. We found no such allele sharing, either between the Iguazú and Uruguay

River species flocks or between them and the Atlantic coast lineages (Fig. 4). We found more signatures of hybridization within the species flocks, between the Iguazú flock and its stem group in the Paraná, and within the Paraná (Fig. 4). These cases of gene flow may explain the discordant positions of these species in the ASTRAL and SNAPP trees as well as the mitonuclear discordance between the Iguazú and Paraná species (Piálek et al. 2019; Říčan et al. 2021). The absence of ancestral hybridization events may explain the comparatively modest extent of the Uruguay and Iguazú River species flocks in terms of species richness relative to the extensive radiations in the East African Great Lakes.

#### Extrinsic Drivers of Adaptive Radiation in Pike Cichlids

Escape from competition is often viewed as a major ecological release that may spark adaptive radiation (Schluter 1988). Fish diversity in the La Plata Basin is not as high as the Amazon, with very reduced genus-level diversity, but lineages often form speciesrich assemblages with high local endemism (Lucena and Kullander 1992; Neves et al. 2015; Piálek et al. 2019). Pike cichlids are one of several cichlid lineages that occur in the La Plata Basin. The others (e.g., Australoheros, *Gymnogeophagus, Cichlasoma, and Apistogramma*) tend to be generalist omnivores that prefer vegetated littoral zones (Yafe et al. 2002). In contrast, pike cichlids are generally predators (Montaña and Winemiller 2009; Burress et al. 2013) and, in the La Plata Basin, many Crenicichla species have a distinct affinity for rocky rapids, especially those species in the two species flocks (Lucena and Kullander 1992; Lucena 2007; Piálek et al. 2015; Serra et al. 2016). Correspondingly, the presence of few incumbent competitors and underutilized resources in the La Plata basin may have set the stage for adaptive radiation of pike cichlids, in a manner analogous to the cichlid adaptive radiations that arose in the East African Great Lakes (Wagner et al. 2012; McGee et al. 2020).

Predation is largely viewed as having an inhibitory effect on diversification (Schluter 1988) by restricting the ability to utilize some resources such as those distributed away from shelter or near resources frequented by predators. However, adaptive radiation can also be driven by selection imposed by predation, evidenced by the parallel diversification of Hawaiian stick spiders (Gillespie et al. 2018), ecological speciation by stick insects (Nosil and Crespi 2006), and adaptive radiation of mainland anoles (Losos 2009; Burress and Muñoz 2021), which utilize dark markings and drab coloration to avoid detection by predators. The parallel adaptive radiations of pike cichlids may have similarly been influenced by predation, as they exhibit similar patterns of counter shading, drab gray and olive base colors, and similar combinations of dark blotches and barring along their back and flanks (Piálek et al. 2019). These pike cichlids are relatively small-bodied (generally less than 30 cm; Lucena and Kullander 1992) and, therefore, at risk of predation from any large predator found in the La Plata Basin including catfishes, characids, otters, and birds. Their diminutive size likely explains their extensive use of eye spots to draw attention away from vital body regions, as an antipredator measure (Hemingson et al. 2020, 2021). Thus, pike cichlids may rely on crypsis to reduce predation risk while foraging on rock-associated prey in shallow, clear waters.

The La Plata Basin itself may have physical attributes favorable for extensive utilization of the benthos. For example, its tributaries tend to be wide, shallow, and full of rocky slabs, which are favorable conditions for algae growth. These conditions make dietary niches in pike cichlids closely linked to algae, both directly (e.g., browsers) and indirectly (e.g., molluscivores), viable evolutionary outcomes of adaptive radiation. The La Plata Basin is also a gastropod biodiversity hotspot (Strong et al. 2007), in large part due to the endemic genus Potamolithus (López Armengol 1985), the dominant food source of the molluscivorous *Crenicichla* in both species flocks (Lucena and Kullander 1992, Casciotta et al. 2013; Piálek et al. 2015; Říčan et al. 2017). Potamolithus is almost entirely endemic to the La Plata Basin (Rumi et al. 2006) and is the largest genus of freshwater snails in Argentina (Rumi et al. 2008) and Uruguay (Scarabino 2004). A subset of the Potamolithus geographic distribution overlaps with a distribution of volcanic rocks (flood basalts of the Paraná formation; Brea and Zucol 2011; Piálek et al. 2019, 2015). This region of overlap is the only place where the pike cichlid radiations evolved (Piálek et al. 2012, 2015, 2019), suggesting that the ample availability of snails and rocky habitats may have been a prerequisite for their adaptive radiation.

## Intrinsic Drivers of Adaptive Radiation in Pike Cichlids

Functional innovations, such as pharyngeal jaws in some fishes (Liem 1973; Burress et al. 2020) and adhesive toe pads in some lizards (Miller and Stroud 2021; Burress and Muñoz 2021), may promote diversification by enhancing access to otherwise inaccessible niches. Several innovations have evolved in the Uruguay and Iguazú River species flocks. Hypertrophied lips, found in Crenicichla tendybaguassu and Crenicichla tuca, are conspicuous adaptations that facilitate feeding from rock crevices and interstitial gaps by creating seals that reduce prev escape potential (Lukas et al. 2015; Henning et al. 2017), thereby permitting the use of spatially restricted resources. Despite being an uncommon phenotype, these hypertrophied, or thicker, lips have conspicuously evolved during cichlid adaptive radiations (Elmer et al. 2010; Colombo et al. 2013; Machado-Schiaffino et al. 2014, 2017; Hulsey et al. 2018). Similarly, hypertrophied pharyngeal jaws, found in Crenicichla minuano and Crenicichla jurubi in the Uruguay River, C. tesay in the Iguazú River, and C. yaha, and C. taikyra in other parts of the Parana River, are necessary to generate sufficient bite force to crush mollusc shells (Hulsey et al. 2008). Crenicichla minuano, C. tesay, and C. yaha have among the most robust pharyngeal jaws among

American cichlids (Burress 2016; Burress et al. 2018b). By contrast, pike cichlid oral jaws are poorly suited to grazing rock surfaces when compared with species with highly modified, compact jaws, and specialized dentition adapted to scraping algae (e.g., Hypsophrys and Tomocichla; Casciotta and Arratia 1993; Winemiller et al. 1995) or reef fishes with numerous innovations that facilitate biting (i.e., modified jaw joints and dentition; Wainwright and Longo 2017; Burress and Wainwright 2019). In pike cichlids, the browsing ecomorph consists of only two modest modifications to the oral jaws-the reduced size of the mouth and slight curvature of the snout to position the mouth downward (Lucena 2007; Piálek et al. 2015). A lack of apparent innovations to facilitate biting or scraping suggests these species merely pick at algae growing from rock surfaces.

Functions, such as the motion of fish jaws during feeding, are emergent properties of structures and may also promote diversification. Pike cichlids exhibit many-toone mapping (Fig. 1) in which many oral jaw phenotypes produce similar mechanical properties (Wainwright et al. 2005). For example, crevice-feeders, molluscivores, and browsers have very differently shaped oral jaws, yet their jaw systems produce similar mechanical output (Fig. 1). Mechanical redundancy may promote phenotypic diversity by avoiding evolutionary constraints imposed by trade-offs (Alfaro et al. 2005). In fishes, there is a trade-off between the ability to transmit force across jaw joints and both the speed and magnitude of jaw movements (Westneat 2004, 2005). In such situations, the evolution of a structural system can be limited following specialization in one function. Pike cichlids generally have velocity-modified oral jaws (Burress et al. 2020; Wainwright et al. 2001) such that the lineage is engaged in a relatively strong mechanical tradeoff. Therefore, many-to-one mapping may be a crucial mechanism by which the group has diversified their jaw systems in a manner that generates a diversity of feeding ecologies while avoiding morphological changes that would exacerbate an underlying mechanical tradeoff. Further, functional innovations of soft tissues, rather than bone elements (e.g., hypertrophied lips) facilitated the exploitation of novel spatially restricted prey from crevices and interstitial gaps, without the necessity of anatomical changes that might result in a mechanical trade-off. Similarly, the fact that the oral jaws are freed from functional demands associated with processing prey, which are instead performed by the pharyngeal jaws, results in some independence of functions associated with prey capture and processing (Liem 1973; Burress et al. 2020; Conith and Albertson 2021; Ronco and Salzburger 2021; but see Burress and Muñoz 2021). Adaptations to the pharyngeal jaws may have provided pike cichlids a means to exploit an array of nonevasive prey that require the generation of force (e.g., molluscs and algae), although requiring relatively small adaptations to the oral jaws. Therefore, many-toone mapping, soft tissue functional innovations, and functional decoupling may operate together to promote

adaptive radiation of pike cichlids by permitting dramatic changes in feeding ecology via structural changes that bypass mechanical trade-offs.

#### **CONCLUSIONS**

Pike cichlids share a major feature—parallel ecomorphological diversification—with several prominent examples of adaptive radiation, including Caribbean anoles (Losos 2009), East African cichlids (Seehausen 2015), and Hawaiian stick spiders (Gillespie et al. 2018). Despite many similarities in the ecological niches and environmental axes exploited by pike cichlids and other fish adaptive radiations, little evidence of gene flow and decoupling of form and function stand out as major departures from patterns shared by other groups (Martinez et al. 2018; Salzburger 2018; Svardal et al. 2021). A mixture of release from the competition, the evolution of several functional innovations, and key physical features of the La Plata Basin likely set the stage for the pike cichlid adaptive radiations.

#### SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: https://doi.org/10.25338/B8XP7D.

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## DATA AVAILABILITY

Data are available from the Dryad Digital Repository (https://doi.org/10.25338/B8XP7D). Sequences are deposited in Sequence Read Archive, National

Center for Biotechnology Information, SRA accession PRJNA705376 (http://www.ncbi.nlm.nih.gov/sra/ PRJNA705376).

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