

# TROPHIC NOVELTY IS LINKED TO EXCEPTIONAL RATES OF MORPHOLOGICAL DIVERSIFICATION IN TWO ADAPTIVE RADIATIONS OF *CYPRINODON* PUPFISH

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Adaptive radiations are known for rapid morphological and species diversification in response to ecological opportunity, but it remains unclear if distinct mechanisms drive this pattern. Here, we show that rapid rates of morphological diversification are linked to the evolution of novel ecological niches in two independent *Cyprinodon* radiations nested within a wide-ranging group repeatedly isolated in extreme environments. We constructed a molecular phylogeny for the Cyprinodontidae, measured 16 functional traits across this group, and compared the likelihoods of single or multiple rates of morphological diversification. We found that rates of morphological diversification within two sympatric *Cyprinodon* clades containing unique trophic specialists are not part of an adaptive continuum with other clades, but are instead extreme outliers with rates up to 131 times faster than other Cyprinodontidae. High rates were not explained by clade age, but were instead linked to unique trophic niches within *Cyprinodon*, including scale-eating, zooplanktivory, and piscivory. Furthermore, although both radiations occur in similar environments and have similar sister species, they each evolved unique trophic specialists and high rates of morphological diversification in different sets of traits. We propose that the invasion of novel ecological niches may be a key mechanism driving many classic examples of adaptive radiation.

**KEY WORDS:** Brownian motion, comparative method, disparity, divergent selection, ecological speciation, lepidophagy.

Adaptive radiations have been central to evolutionary thinking since its inception (Darwin 1839; Gavrillets and Losos 2009). Classic examples of adaptive radiation, such as the Galapagos finches, East African cichlids, Hawaiian silverswords, and Caribbean *Anolis*, not only helped inspire the theory of natural selection, but have also provided important case studies of ecology and evolution (Seehausen 2006; Grant and Grant 2007; Losos 2009). In addition to these well-known and spectacular adaptive radiations that we refer to as “classic,” many new groups are now also considered adaptive radiations based on Schluter’s (2000) influential criteria for defining adaptive radiation: common ancestry, adaptive trait divergence in response to divergent environments, and

rapid speciation. This approach emphasizes adaptation at the level of closely related species due to divergent ecological selection in the presence of ecological opportunity (Schluter 2000; Losos and Mahler 2010; Yoder et al. 2010). Divergent selection for resource use may result in increased intraspecific variation (e.g., Parent and Crespi 2006) or ecological diversification into a variety of species adapted to different niches (Losos 2010; Yoder et al. 2010).

Renewed study of ecology’s role in the speciation process since Schluter’s review has found that most speciation events are driven to some extent by ecological factors and can be called adaptive, regardless of whether speciation occurs in the presence of gene flow or in allopatry (Sobel et al. 2010; Price 2008; but

see e.g., Roberts et al. 2009; Sauer and Hausdorf 2009). Thus, adaptation appears to be rampant among newly formed species and there is still no consensus on what constitutes “rapid speciation.” In practice, this has resulted in the identification of nearly any clade as an adaptive radiation, leading some to propose that the term has lost all meaning (Olsen and Arroyo-Santos 2010) or does indeed apply to every adaptively diversifying clade (Glor 2010).

There have been some attempts to push back against this expanding definition of adaptive radiation by measuring diversification rate within a focal clade relative to outgroups (Givnish 1997; Losos and Miles 2002; Harmon et al. 2003). In contrast to Schluter’s focus on ecological divergence mechanisms, these comparative approaches often derive from Simpson’s (1953) pattern of adaptive radiation as “more or less simultaneous divergence of numerous lines from much the same ancestral adaptive type.” Several new phylogenetic comparative methods now facilitate rigorous comparisons of lineage and morphological diversification rates among clades, leading to new comparative definitions of adaptive radiation (e.g., Harmon et al. 2003; O’Meara et al. 2006; Rabosky and Lovette 2008; Agrawal et al. 2009; Harmon et al. 2010). However, these approaches sometimes draw arbitrary thresholds across a continuum of adaptively diversifying clades (Olsen and Arroyo-Santos 2010). Although they provide quantitative thresholds for identifying adaptive radiations, it is not always clear if these thresholds are biologically meaningful.

Neither of these two broad conceptual approaches to the study of adaptive radiation distinguishes among different types of ecological divergence. In Schluter’s framework, any type of rapid, ecologically derived reproductive isolation is treated no differently than the most spectacular examples of ecological diversification within many classic examples of adaptive radiation (e.g., Arbogast et al. 2006). Similarly, comparative phylogenetic methods use morphological or species diversity, but not ecological diversity, to define adaptive radiations.

However, not all adaptation is the same. Adapting to a similar niche in a new environment may occur rapidly and drive increased reproductive isolation as a byproduct, fulfilling existing criteria for adaptive radiation, but this may be a qualitatively different process than adapting to a novel niche. Ecological novelty is a rare and distinctive type of adaptation, but we observe that it is a common feature of many classic examples of adaptive radiation in isolated, largely competitor-free environments. Within these radiations, species often specialize on novel resources compared to the resources used by species in a larger paraphyletic outgroup. For example, blood-drinking, plant-eating, and wood-probing insectivory are novel niches occupied by different species of Galapagos finches, whereas all other domed-nest building finches are granivores (Burns et al. 2002; Grant and Grant 2007). The other lineages of domed-nest finches exhibit a simi-

lar degree of morphological disparity and have each adapted to granivore niches in new island environments (Burns et al. 2002), but only the Galapagos finches have grossly expanded beyond their normal resource range. Thus, trophic novelty is one of the factors that distinguishes this classic adaptive radiation from other island finches, not only morphological disparity or ecologically derived reproductive isolation. There are many similar examples of substantial ecological novelty within well-known adaptive radiations relative to their larger paraphyletic outgroups that display little ecological diversity despite also adapting to new habitats (e.g., Fryer and Iles 1972; Lovette et al. 2002; Givnish et al. 1997).

Here, we tested the hypothesis that ecological novelty is associated with extreme rates of morphological diversification within the Cyprinodontidae, a wide-ranging group of fishes that has repeatedly speciated within isolated habitats, (Miller et al. 2005). Rapid ascent of novel fitness peaks during adaptation to novel ecological niches could drive the rapid rates of morphological diversification that are commonly observed within adaptive radiations (e.g., Ackerly 2009), exceeding morphological diversification rates between highly similar niches that require smaller phenotypic changes. We argue that extreme differences in morphological diversification rates across *Cyprinodon* clades reflect biologically meaningful differences between adapting to novel trophic niches and adapting to similar trophic niches in new environments.

We measured relative rates of morphological diversification in two independent clades of sympatric *Cyprinodon* pupfishes that appear to fall outside the ordinary adaptive continuum of Cyprinodontidae. Each clade contains sympatric species occupying specialized trophic niches unique among all species of *Cyprinodon*, including a piscivore and a planktivore in Lake Chichancanab, Mexico (Stevenson 1992; Horstkotte and Strecker 2005) and a scale-eater and hard-shelled prey specialist on San Salvador Island in the Bahamas (Martin and Wainwright 2009). To our knowledge, this is the only scale-eating specialist among all 1500 species of atherinomorphs (Sazima 1983; Setiamarga et al. 2008). No other piscivore, planktivore, or molluscivore is found among the 55 known species of *Cyprinodon*. Overall, *Cyprinodon* populations have repeatedly speciated in a variety of extreme environments and show corresponding trait variation; thus, this entire clade could be considered an adaptive radiation under existing criteria. However, only the two sympatric clades contain species that occupy novel trophic niches and thus share the rare feature of ecological novelty with many classic examples of adaptive radiation.

We measured 16 functional traits for 48 species of Cyprinodontidae, including both sympatric *Cyprinodon* clades, and constructed an ultrametric phylogenetic tree for the Cyprinodontidae using a supermatrix of five mitochondrial genes. We then

examined the position of the sympatric clades in the Cyprinodontidae morphospace and compared rates of morphological diversification across all young clades of *Cyprinodon* and between trophic specialists and generalists.

## Materials and Methods

### STUDY SYSTEM

*Cyprinodon* is distributed throughout the North American Atlantic coast and Caribbean with many more species and some outgroups (e.g., *Cualac*, *Megupsilon*) restricted to isolated interior water bodies (e.g., Devil's Hole) and additional outgroups in the Mediterranean (*Aphanius*, *Valencia*) and Andes regions (*Orestias*; Hrbek and Meyer 2003; Echelle et al. 2005). All *Cyprinodon* species occur in allopatry except for two sympatric clades (some natural hybrid zones are also known, e.g., Carson and Dowling 2006). Thus, lineage diversification in this group has been largely driven by geographic vicariance events. Across their range *Cyprinodon* occur in a wide variety of habitats, from isolated springs, to rivers, lakes, and marine estuaries and show corresponding trait variation, for example, in the presence/absence of pelvic fins as well as caudal peduncle length, suggesting adaptation to different flow regimes (Miller et al. 2005; C. H. Martin, pers. obs.). Nearly all *Cyprinodon* have a diet composed mainly of algae and detritus. We verified this with an exhaustive literature search of all studies with dietary data or observations of *Cyprinodon* (e.g., Cox 1972; Davis 1981; Naiman 1975; Naiman 1979; Minckley and Minckley 1986; Martinez-Aquino and Aguilar-Aguilar 2008; Stoike and Pister 2010; Carson 2009) as well as information from FishTraits (Frimpong and Angermeier 2009; Miller et al. 2005).

There are only two known exceptions to this pattern of allopatric detritivores: Lake Chichancanab, Mexico (Humphries and Miller 1981) and the interior lakes of San Salvador Island, Bahamas (Holtmeier 2001; Turner et al. 2008). Each system contains closely related *Cyprinodon* species that occur in sympatry and have specialized on a diverse range of resources. Both systems are large, shallow (1–13 m), isolated basins of brackish or hypersaline water with remarkably similar and depauperate fish communities. At least five *Cyprinodon* species originally coexisted in Lake Chichancanab with only one other fish species, *Gambusia sexradiata* (Humphries and Miller 1981; Humphries 1984). This included the largest *Cyprinodon* species, *C. maya*, the only species in the lake which included fish in its diet (Stevenson 1992; Horstkotte and Strecker 2005) and the second smallest *Cyprinodon* species, *C. simus*, which fed on zooplankton in large schools (Humphries and Miller 1981; Stevenson 1992). Two more species, *C. labiosus* and *C. verecundus*, fed predominantly on amphipods and bivalves, respectively, and a fifth species, *C. beltrani*,

fed on algae and detritus and was similar in both morphology and ecology to the coastal sister species to the radiation, *C. artifrons* (Horstkotte and Strecker 2005; Miller et al. 2005). Two additional species were also recently described, *C. esconditus* and *C. sauviuum* (Strecker 2002, 2005), but their ecology is unknown and we were able to obtain only one of these species for measurement due to their rarity in museum collections. The Chichancanab species appear functionally specialized for their specialized trophic niches; the piscivore has proportionally larger jaws and adductor muscles whereas the zooplanktivore has narrow, highly protrusible jaws with short teeth for increased suction flows. Three of the five originally described species are now essentially extinct in the wild after the introduction of *Oreochromis mossambicus* and *Astyanax fasciatus* (Schmitter-Soto and Caro 1997; Strecker 2006b; C. H. Martin, pers. obs. 2009). Only the detritivore, *C. beltrani*, and the amphipod-eater, *C. labiosus*, still occur in the lake at detectable frequencies (C. H. Martin, pers. obs. 2009).

Three undescribed *Cyprinodon* species coexist in the hypersaline interior lakes on San Salvador Island with two other fish species, *Gambusia hubbsi* and the surface-dwelling silver-side *Atherinomorus stipes* (Holtmeier 2001; Turner et al. 2008). One *Cyprinodon* species is a generalist detritivore similar in morphology and ecology to the wide-ranging coastal sister species to the radiation, *C. variegatus*. Another species has a larger in-lever to out-lever ratio for closing its lower jaw with more force and a protruding, reinforced nasal region probably used for crushing its specialized diet of ostracods and gastropods, comprising 30% of its stomach contents ( $N = 63$ ; Martin and Wainwright 2009). The third species has enlarged adductor muscles, smaller in-lever to out-lever ratio for rapidly closing its large lower jaw, and an elongated body for quickly performing scale-removing strikes on other *Cyprinodon* and *Gambusia*. A total of 50% of the stomach contents in this species were scales, while no individuals had consumed whole fish ( $N = 60$ ; Martin and Wainwright 2009). All three species occur in at least two different isolated lake systems on the island with reduced gene flow among lakes (Turner et al. 2008).

Reproductive isolation among species in both sympatric *Cyprinodon* clades is supported by significant genetic differentiation in microsatellite allele frequencies (Strecker 2006a; Turner et al. 2008) and field and laboratory observations of assortative mating (Strecker and Kodric-Brown 2000; C. H. Martin, unpubl. data). Furthermore, common garden experiments support the genetic basis of species phenotypes in both radiations (Holtmeier 2001; C. H. Martin, unpubl. data).

Both sympatric clades may be very young. Sediment cores date the age of Lake Chichancanab to be  $8000 \pm 200$  years (Covich and Stuiver 1974; Hodell et al. 1995; Supporting information) and changes in sea-level suggest that the interior lakes of San Salvador Island were dry from 15,000 to 6,000 years ago

(Pacheco and Foradas 1986; Milliken et al. 2008; Supporting information). However, age estimates based on the mitochondrial diversity in each clade predate these geographic ages so we have conservatively based our analyses only on the age estimates from the molecular data. Due to the possibility that this mitochondrial diversity predates the divergence of these species as a result of mitochondrial introgression or ancestral polymorphism (e.g., Shaw 2002; Mims et al. 2010), we have also included a supplemental analysis of morphological diversification rates based on phylogenies calibrated to the geographic ages of these lakes and additional fossil and geographic priors (Supporting information).

### PHYLOGENETIC ANALYSES

We assembled molecular data for 43 species or subspecies of *Cyprinodon* (out of 52 named species or subspecies plus the three undescribed species on San Salvador Island), 12 populations of *C. variegatus*, and 15 outgroup taxa, including *Megupsilon*, *Cualac*, *Floridichthys*, *Jordanella*, *Garmanella*, *Orestias*, *Valencia*, and *Aphanius* from previously published studies (Hrbek and Meyer 2003; Echelle et al. 2005; Echelle et al. 2006; Haney et al. 2007; Parker and Kornfield 1995). We used PhyLoTA (Sanderson et al. 2008) to download five mitochondrial gene regions (Table S1) from GenBank and constructed a 6683 base pair supermatrix for these 69 taxa. Sequence information was available for approximately 33% of this matrix with at least 11 overlapping taxa within each gene region (exceeding the recommendations of Thomson and Shaffer 2010). The 50% majority consensus sequence was used for taxa represented by more than one sequence in GenBank. Sequences were aligned using MUSCLE (Edgar 2004) and then adjusted manually as needed. All five gene regions were concatenated into a single matrix using Geneious (version 4.8.5; Drummond et al. 2006).

Phylogenetic hypotheses were generated using Bayesian Markov Chain Monte Carlo (MCMC) sampling. We used BEAST (version 1.6.0; Drummond and Rambaut 2007) to estimate a posterior distribution of ultrametric phylogenetic trees based on the mitochondrial supermatrix. We used a relaxed clock model of sequence evolution under an uncorrelated lognormal distribution and a Yule prior for the speciation process. We fixed the mean clock rate to 1.0 to generate ultrametric trees with branch lengths in relative time units. We also performed the analyses using fossil and geographic priors on the ages of 6 nodes in the tree (Supporting information; see earlier discussion in Study System). Our key results were robust to analyses based on either method of dating the tree.

Five alternative partitioning schemes were compared using Bayes factors (Table S1), and we used the best-fit scheme containing a separate partition for each noncoding gene region (16S rRNA, tRNA<sup>Leu,Ile,Met</sup>, and control region) and a separate partition for each codon position in coding genes (ND2 and cytochrome

b) with independent substitution rate and rate heterogeneity parameters. We assigned the best model of nucleotide substitution for each partition from their relative AIC scores using jModelTest (Posada 2008; Guindon and Gascuel 2003; Table S1).

We assessed the convergence and mixing of six independent MCMC runs of at least seven million generations using Tracer (version 1.6.0; Drummond and Rambaut 2007) and discarded the first three million generations in each run as burn-in or the number of generations needed to reach stationarity. All six runs converged on the same stationary distribution. We combined the stationary distributions using LogCombiner (version 1.6.0) for a total chain length of 39 million generations; all parameters had an effective sample size greater than 340.

### MORPHOLOGICAL TRAITS

We measured 16 morphological traits and standard length for 48 taxa in our tree, spanning the phylogeny of Cyprinodontidae (Fig. 1). A median of three specimens (mean = 2.9) was measured per taxon, depending on the availability of specimens (Appendix S1). We selected traits with specific connections to foraging performance (Hernandez et al. 2009; Wainwright et al. 2004) and features that appeared to clearly differ across the Cyprinodontidae. The following numbered traits correspond to the labels in Figure 3 and Table 1. For each specimen, we first dissected divisions A1–3 of the adductor mandibulae complex (AM) from both sides of the head. (1) A1 was weighed separately from (2) A2 and A3. Muscle masses were weighed to the nearest 0.0001 g and then cube root transformed for dimensional equivalence. Next, each specimen was cleared and double-stained with alizarin red and alcian blue for bone and cartilage. Thirteen linear measurements were made by a single observer (CHM) using an ocular micrometer and dissecting microscope. (3) Lower jaw length was measured from the jaw joint to the tip of the most anterior tooth. (4) The jaw closing lever was measured from the jaw joint to the midpoint of the attachment of the AM on the articular coronoid process. (5) The jaw opening lever was measured from the jaw joint to the attachment midpoint of the interopercular mandibular ligament on the angular bone. The premaxilla was divided into in-lever and out-lever components based on its function in jaw protrusion within Cyprinodontiformes; contraction of the A1 pulls the maxilla and the lower in-lever of the premaxilla in a posterior direction, rotating the upper out-lever of the premaxilla forward and increasing jaw protrusion (Hernandez et al. 2009). (6) The in-lever of the premaxilla was measured from the ventral tip of the premaxilla to the center of rotation midway along the premaxilla marked by an area of greater ossification. (7) The out-lever of the premaxilla was measured from the center of rotation to the tip of the most anterior tooth on the premaxilla. (8) The maxilla was measured from the ventral tip to its point of rotation at the palatine sulcus marked by a cartilaginous region on the

palatine. (9) The anterior–posterior length of the maxillary external process was measured in dorsal view. (10) Gape was measured from the distance between the articular bones anterior to the jaw joint in the ventral view. (11) Jaw protrusion was measured by maximally depressing the lower jaw and measuring the distance from the tip of the most anterior tooth on the premaxilla to the premaxillary sulcus on the maxilla. (12) The number of tooth positions was counted on the dentary. (13) Tooth length was measured from the distance from the root to the tip of the first fully emerged tooth on the premaxilla in the lateral view. (14) Orbit was measured from the horizontal diameter of the orbit in the lateral view. (15) Cranial width was measured from the minimum width of the supraorbital neurocranium in the dorsal view. Finally, we used dial calipers to measure the (16) maximum body depth and standard length (SL) of each specimen.

All measurements were averaged for each species. To remove the effects of size, we used the residuals from a linear regression of natural log-transformed trait on natural log-transformed SL using species averages. This procedure controls for unequal sampling among species and allometric scaling with SL. We performed a phylogenetically corrected principal component analysis (Revell 2009) on all size-corrected residuals to examine the position of each species in the Cyprinodontidae morphospace. To quantify morphospace occupation, we compared the variance of each sympatric clade to the distribution of variances of randomly selected groups of allopatric *Cyprinodon* species of the same size on each of the first eight principal component axes, explaining 94.2% of the total variation in the dataset.

Second, to measure the total magnitude of divergence within the morphospace, we calculated the distance between each sympatric species and its nearest allopatric sister species on the first eight principal component axes. The allopatric sister species to each sympatric clade are wide-ranging coastal detritivores and due to their morphological similarity across their range, they present a good estimate for the morphology of the ancestral founding population of each sympatric clade. We compared the divergence of sympatric species to the distribution of divergences between each allopatric sister species and all other allopatric *Cyprinodon* species. This generates a null distribution of allopatric divergence distances to compare the divergence of each sympatric *Cyprinodon* species.

Finally, to isolate the effect of trophic specialization on the total magnitude of divergence in morphospace, we calculated the mean distance between each trophic specialist (San Salvador  $n = 2$ ; Chichancanab  $n = 4$ ) and the nearest allopatric sister species for both sympatric clades. We compared this mean divergence distance for trophic specialists to the null distribution of mean allopatric divergence distances in a random *Cyprinodon* group of the same size (i.e., San Salvador  $n = 2$ ; Chichancanab  $n = 4$ ).

## MORPHOLOGICAL DIVERSIFICATION RATES

We used BROWNIE (version 2.1; O'Meara et al. 2006) to calculate the likelihood of the observed morphological diversity across the estimated phylogenies under a Brownian motion model. Brownian motion is a simple random-walk process and only applies to adaptive evolution under certain restricted conditions (Hansen et al. 2008; O'Meara et al. 2006); however, the model is directly applicable to the macroevolutionary prediction of adaptive radiation: morphological variance should increase at a faster rate within an adaptive radiation relative to outgroups (e.g., Losos and Miles 2002). For each rate test, we compared one of the sympatric *Cyprinodon* clades to all other Cyprinodontidae in our tree while excluding the other sympatric clade. For example, we fit a two-rate model with one rate estimated for the Chichancanab clade and a second independent rate estimated for all other Cyprinodontidae with the San Salvador radiation excluded and vice versa. We compared the likelihood of the data under the one- and two-rate models using the Akaike information criterion with a correction for small sample sizes ( $AIC_c$ ) and considered a difference in scores  $\geq 8$  to strongly favor one model over another (Burnham and Anderson 2002). The relative rate of morphological diversification was the ratio of the focal group's rate to the background Cyprinodontidae rate under the two-rate model. To account for phylogenetic uncertainty, we took the average rates and  $AIC_c$  scores from a sample of 100 trees drawn from the stationary distribution of the combined MCMC sample (Huelsenbeck et al. 2000). One tree was sampled every 390,000 steps in the chain to minimize autocorrelation. Standard error of the mean rates and  $AIC_c$  scores was calculated from the variance of this sample (similar to the approach of Mahler et al. 2010).

We used a censored rate test for these comparisons because this approach does not make an inference about the internal branch connecting the focal clade with the rest of the tree and calculates the two rates of morphological diversification independently (O'Meara et al. 2006). The two sympatric clades in this study each presumably had a discrete beginning in time after first colonizing their respective lakes. The censored rate test is ideal for this situation because it models a discrete rate shift, rather than a gradual rate change along the internal branch leading up to the root node of each clade.

We also compared relative morphological diversification rates across all young *Cyprinodon* clades to determine if the sympatric clades were exhibiting an unusual pattern among *Cyprinodon*. In addition to the two sympatric clades, we calculated relative morphological diversification rates for all two-species ( $n = 9$ ), three-species ( $n = 6$ ), and four-species ( $n = 3$ ) allopatric *Cyprinodon* clades relative to all other Cyprinodontidae as described previously with both sympatric clades removed. Using BROWNIE, we estimated diversification rates on the first and second principal component axes of the Cyprinodontidae

morphospace and calculated standard error due to phylogenetic uncertainty.

To account for the shared history of these clades, we then compared the observed pattern of relative morphological diversification rates to the null expectation by simulating character evolution under the Brownian motion model on the maximum sum of clades tree (Fig. 1). We used the *ape* (Paradis et al. 2004) and *geiger* packages (Harmon et al. 2008) in R (R Development Core Team 2010) to simulate Brownian evolution of all 16 size-corrected traits within the constraints imposed by the observed variances and covariances among traits. After each simulation, we recalculated the phylogenetically corrected morphospace and then calculated the relative rate of morphological diversification on the first and second principle component axes for every *Cyprinodon* clade as described previously. We compared the observed rates of morphological diversification with the null distribution of rates of morphological diversification across all nested *Cyprinodon* clades from 1000 simulations.

Finally, to isolate the effect of trophic novelty on morphological diversification rates, we used the noncensored rate test in BROWNIE to compare the likelihood of a two-rate model with one rate for trophic specialists (Chichancanab  $n = 4$ ; San Salvador  $n = 2$ ) and a second rate for all detritivorous *Cyprinodon* (including each detritivore in the two sympatric clades) to a model with a single rate of morphological diversification for both dietary categories using  $AIC_c$ . We estimated morphological diversification rates on the first eight principal component axes. We used SIMMAP version 1.0 (Bollback 2006) to simulate 500 ancestral state reconstructions for the binary trait of diet (trophic specialist or detritivore) while sampling across the 100 trees drawn from the MCMC sample. Due to inadequate dietary information, all outgroups to *Cyprinodon* were excluded as well as the recently described Chichancanab species *C. esconditus*.

## Results

### CYPRINODONTIDAE PHYLOGENY

Our phylogeny was largely congruent with previous hypotheses for this group (Hrbek and Meyer 2003; Echelle et al. 2005; Echelle et al. 2006; Haney et al. 2007) and most nodes were resolved with posterior probabilities greater than 0.95 (Fig. 1). In particular, we found support for the monophyly of both sympatric radiations with a posterior probability of 1 (Fig. 1).

Our analyses of morphological diversification rates were robust to phylogenies calibrated to absolute time (using fossil and geographic prior information [Table S2]) or relative time (using only mitochondrial sequence data). All results presented here used phylogenies calibrated in relative time to be conservative; estimated relative morphological diversification rates in the two sympatric clades were up to 29 times higher using absolute time-

calibrated phylogenies for our analyses (Table S3). However, the ages of young clades estimated from two fossil priors were older than the geographic priors for these clades. These conflicting priors caused high autocorrelation of the MCMC sample and long run lengths were necessary to achieve adequate effective sample sizes (Supporting information).

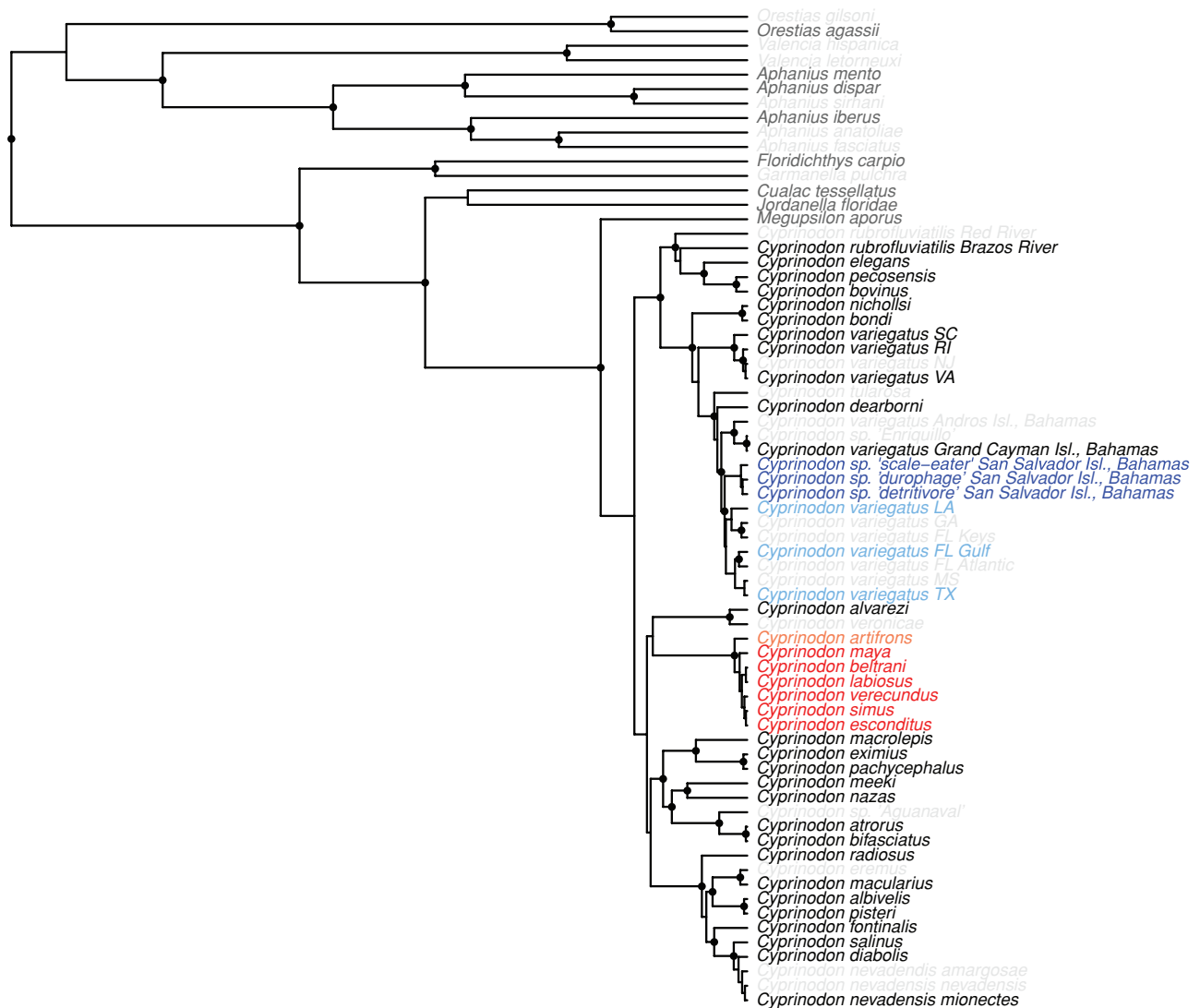
### CYPRINODONTIDAE MORPHOSPACE

The first two principal component axes of the phylogenetically corrected morphospace explained 52.1% of the total variance (Table S4, Fig. 2). PC1 mainly described decreasing overall jaw size, including maxilla length, jaw length, closing lever, A1, and lower and upper premaxilla length (Table S4). PC2 mainly described increasing tooth length and decreasing cranial width, opening lever, and maxillary process (Table S4). The first eight axes explained 94.2% of the total variance for the 16 size-corrected residual trait values. The total variance of both the Chichancanab clade and the San Salvador clade was significantly higher on the first principal component axis than allopatric *Cyprinodon* (Chichancanab:  $P = 0.006$ ; San Salvador:  $P = 0.019$ ). The total variance of the San Salvador clade was also marginally higher than allopatric *Cyprinodon* on the third principal component axis ( $P = 0.058$ ).

The total magnitude of morphological divergence on the first eight principal component axes was significantly larger for several trophic specialists, including the scale-eater ( $P < 0.0001$ ), hard-shelled prey specialist ( $P = 0.041$ ), piscivore ( $P = 0.032$ ), and marginally larger for the zooplanktivore ( $P = 0.067$ ), relative to their nearest allopatric sister species when compared to the distribution of distances to other allopatric *Cyprinodon* species in the phylogenetically corrected morphospace. Within each sympatric clade, the total morphological divergence between the sympatric detritivores and their nearest allopatric sister species was smaller than the distance to any trophic specialist. In contrast, the mean morphological divergence of trophic specialists relative to their nearest allopatric sister species was significantly greater than allopatric *Cyprinodon* species (San Salvador trophic specialists:  $n = 2$ ,  $P < 0.0001$ ; Chichancanab trophic specialists:  $n = 4$ ,  $P = 0.005$ ).

### MORPHOLOGICAL DIVERSIFICATION RATES IN THE TWO SYMPATRIC RADIATIONS

A higher rate of morphological diversification was strongly supported in the Chichancanab clade relative to all other Cyprinodontidae for 14 of 16 traits measured (Table 1, Fig. 3;  $\Delta AIC_c > 8$ ). Similarly, higher rates in the San Salvador clade were strongly supported for seven of 16 traits (Table 1, Fig. 3). Although the relative rates were high in both sympatric clades, rates of morphological diversification were not correlated between the same traits in the two clades (Fig. 3;  $r^2 = 0.081$ ,  $P = 0.225$ ).



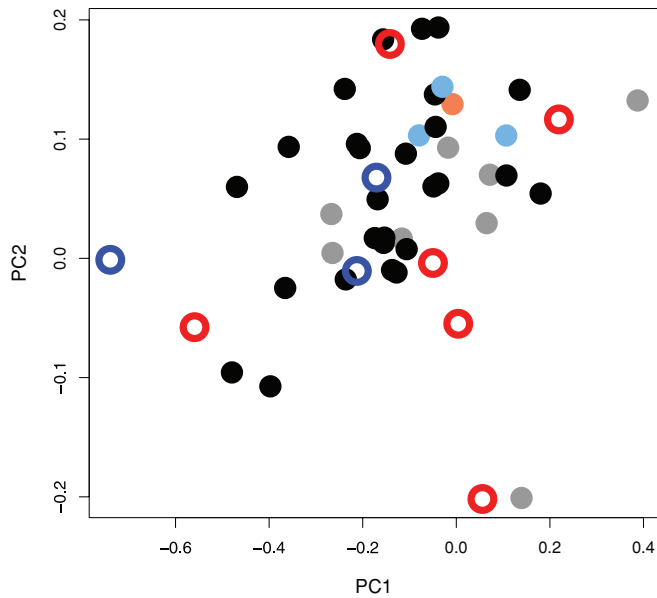
**Figure 1.** Maximum sum of credible clades phylogeny for the Cyprinodontidae with branch lengths in units of relative time. Nodes supported by a posterior probability  $\geq 0.95$  are indicated with a black dot. The Chichancanab clade is shown in red, the San Salvador clade in blue, the nearest allopatric sister species to each sympatric clade in lighter shades of red and blue, respectively, additional measured *Cyprinodon* in black, measured outgroups in dark gray, and unmeasured taxa included in phylogenetic analyses in light gray. Phylogenies were estimated under a relaxed molecular clock with a fixed mean clock rate using BEAST.

The fastest rate in the Chichancanab clade was tooth length, diversifying 131 times faster than other Cyprinodontidae (Table 1, Fig. 3). Equivalently, the Chichancanab clade would need to have diverged over a time period 131 times longer than its estimated age to have rates equal to other Cyprinodontidae. If we take the estimated relative age of Chichancanab at 0.0043, this time period for equivalent rates is longer than the root node of Cyprinodontidae (0.4125), which is at least 21 million years old (Reichenbacher and Kowalke 2009; Supporting information). The fastest rate in the San Salvador clade was the length of the upper premaxilla, diversifying 51 times faster than other Cyprinodontidae (Table 1, Fig. 3). Similarly, using the estimated relative

age of 0.0035, the San Salvador clade would need to have diverged over a time period more than twice the age of the entire *Cyprinodon* + *Megupsilon* clade (0.0826; at least 4 million years old) to show equivalent rates.

#### MORPHOLOGICAL DIVERSIFICATION RATES ACROSS ALL YOUNG CYPRINODON CLADES

There was no association between clade age and relative morphological diversification rate on the first two principal component axes (Fig. 4A,B). The two sympatric *Cyprinodon* clades showed extreme outlying rates of morphological diversification on the



**Figure 2.** First two principal components of morphological variation (50.3% of total variation) in the phylogenetically corrected morphospace for 16 size-corrected traits among Cyprinodontidae, including the Lake Chichancanab radiation (○), San Salvador Island radiation (○), sister species to the Chichancanab clade (●), sister species to the San Salvador Island clade (●), all other *Cyprinodon* species (●), and outgroups (●).

first two principal component axes representing over 50% of the total morphological variation (Fig. 4C). Observed rates in both sympatric clades were significantly higher on PC1 than rates simulated under the Brownian motion model on the maximum sum of clades phylogeny for any *Cyprinodon* clade (Fig. 4A;  $P < 0.0001$ ). Observed rates in the Chichancanab clade were also significantly higher on PC2 than simulated rates for any *Cyprinodon* clade (Fig. 4B,  $P < 0.0001$ ; San Salvador clade:  $P = 0.178$ ).

Three allopatric clades also showed significantly higher relative morphological diversification rates relative to rates simulated under Brownian motion. The *eximius* + *pachycephalus* clade exhibited the third fastest rate on PC1 (Fig. 4A;  $P < 0.001$ ). The *bifasciatus* + *atrorus* ( $P = 0.002$ ) and *diabolis* + *nevadensis amargosae* ( $P = 0.025$ ) clades exhibited the second and third fastest rates on PC2, respectively (Fig. 4B).

#### MORPHOLOGICAL DIVERSIFICATION RATES IN TROPHIC SPECIALISTS AND NONSPECIALISTS

Higher rates of morphological diversification were strongly supported for trophic specialist *Cyprinodon* relative to nonspecialist detritivore *Cyprinodon* on six of the first eight principal component axes (Table 2;  $\Delta AIC_c > 8$ ). Trophic specialists in the two sympatric clades diversified up to 19 times faster

than detritivores in the phylogenetically corrected morphospace (Table 2).

### Discussion

We have demonstrated that the evolution of novel trophic niches in two sympatric *Cyprinodon* clades corresponds to unique positions in functional morphospace and rates of morphological diversification orders of magnitude higher than other young *Cyprinodon* clades adapting to similar isolated environments. We argue that this link between ecological novelty and disparate morphological diversification rates is a common feature of many classic adaptive radiations and reflects the invasion of novel fitness peaks on the adaptive landscape.

The two sympatric *Cyprinodon* radiations showed exceptional rates of morphological diversification for most functional traits measured (Table 1, Fig. 3), much higher than rate estimates for similar or identical functional traits in other fish radiations (Hulsey et al. 2010; Price et al. 2010; Collar et al. 2009). These high rates were not due to the young age of these clades, in contrast to a recent meta-analysis of size and shape diversification rates among a large number of radiations (Harmon et al. 2010). Rather, these two sympatric clades are remarkable outliers in their rates of morphological diversification and do not form a continuum with rates in other *Cyprinodon* clades (Fig. 4C). Three other clades also exhibited significantly faster rates of morphological diversification than expected from simulations of Brownian motion on the first or second principal component axes (Fig. 4). Two of these clades contain known hybrid zones (*C. bifasciatus* + *C. atrorus*: Carson and Dowling 2006; *C. pachycephalus* + *C. eximius*: Minckley and Minckley 1986) where mitochondrial introgression (confirmed between *bifasciatus* and *atrorus*, Carson and Dowling 2006) could result in an underestimate of clade age and thus an overestimate of their relative rate of morphological diversification. In the third clade (*C. diabolis* + *C. nevadensis amargosae*), the observed morphological disparity may be due to a considerable effect of phenotypic plasticity in an extreme environment (Lema and Nevitt 2006). In contrast, the morphology of species in both sympatric radiations raised in a common garden indicates that the contribution of phenotypic plasticity to species differences in these radiations is negligible (Holtmeier 2001; C. H. Martin, unpubl. data).

The Cyprinodontidae as a whole have had numerous opportunities for rapid adaptation to new environments. This is a wide-ranging group that has repeatedly adapted to extreme environments, including the hottest temperature in which any vertebrate is known to complete its life cycle (Minckley and Minckley 1986: 43.8°C) and the smallest endemic range of any known vertebrate (Deacon et al. 1995). Thus, we speculate that many *Cyprinodon* species have probably experienced rapid adaptation following the



**Table 1.** Rates (mean  $\pm$  SE) of morphological diversification within two sympatric *Cyprinodon* clades from Lake Chichancanab, Mexico and San Salvador Island, Bahamas. Values are maximum likelihood estimates of the Brownian motion rate parameter calculated separately within each clade and within all other Cyprinodontidae (background rate). Means and standard errors were calculated by repeating rate analyses for 100 trees sampled from the stationary MCMC distribution. The difference in AIC<sub>c</sub> scores is provided for the fit of the two-rate Brownian motion model relative to the one-rate model; negative scores indicate the one-rate model is favored. Relative rates for strongly supported two-rate models ( $\Delta$ AIC<sub>c</sub> > 8) are highlighted in bold. Numbers correspond to the labeled diagram in Figure 3 and definitions of the landmarks in the methods.

Trait	Chichancanab rate	Background rate	Relative rate	$\Delta$ AIC <sub>c</sub>
1. A1	8.625 $\pm$ 2.348	0.074 $\pm$ 0.003	<b>119.812 <math>\pm</math> 28.240</b>	72.644 $\pm$ 3.468
2. A2 & A3	3.279 $\pm$ 0.674	0.061 $\pm$ 0.001	<b>55.020 <math>\pm</math> 11.137</b>	52.217 $\pm$ 2.895
3. Jaw length	12.030 $\pm$ 1.933	0.584 $\pm$ 0.013	<b>20.385 <math>\pm</math> 2.970</b>	27.775 $\pm$ 2.299
4. Opening lever	34.887 $\pm$ 7.431	1.950 $\pm$ 0.037	<b>17.625 <math>\pm</math> 3.472</b>	22.748 $\pm$ 2.270
5. Closing lever	36.295 $\pm$ 6.616	1.630 $\pm$ 0.046	<b>21.966 <math>\pm</math> 3.507</b>	27.654 $\pm$ 2.540
6. Jaw protrusion	21.824 $\pm$ 3.257	1.709 $\pm$ 0.061	<b>13.217 <math>\pm</math> 1.967</b>	17.170 $\pm$ 2.316
7. Gape	27.691 $\pm$ 4.971	5.105 $\pm$ 0.148	5.339 $\pm$ 0.773	6.380 $\pm$ 1.193
8. Maxilla	45.328 $\pm$ 10.798	1.041 $\pm$ 0.022	<b>42.280 <math>\pm</math> 8.542</b>	43.315 $\pm$ 2.886
9. Lower premaxilla	32.449 $\pm$ 12.394	2.006 $\pm$ 0.057	<b>17.082 <math>\pm</math> 6.514</b>	15.480 $\pm$ 2.418
10. Upper premaxilla	15.943 $\pm$ 1.719	0.575 $\pm$ 0.012	<b>28.394 <math>\pm</math> 3.031</b>	37.520 $\pm$ 2.477
11. Maxillary process	25.364 $\pm$ 7.966	1.433 $\pm$ 0.077	<b>17.117 <math>\pm</math> 4.110</b>	20.650 $\pm$ 2.276
12. Number of teeth	14.377 $\pm$ 2.143	1.259 $\pm$ 0.027	<b>11.542 <math>\pm</math> 1.683</b>	15.946 $\pm$ 2.013
13. Tooth length	162.133 $\pm$ 25.621	1.367 $\pm$ 0.042	<b>131.186 <math>\pm</math> 22.207</b>	77.436 $\pm$ 3.570
14. Orbit width	4.827 $\pm$ 1.125	0.628 $\pm$ 0.022	<b>8.117 <math>\pm</math> 1.755</b>	9.815 $\pm$ 1.677
15. Cranial width	22.231 $\pm$ 3.956	0.978 $\pm$ 0.022	<b>23.350 <math>\pm</math> 4.122</b>	28.741 $\pm$ 2.65
16. Body depth	9.447 $\pm$ 4.244	0.961 $\pm$ 0.034	8.854 $\pm$ 3.203	7.761 $\pm$ 1.911

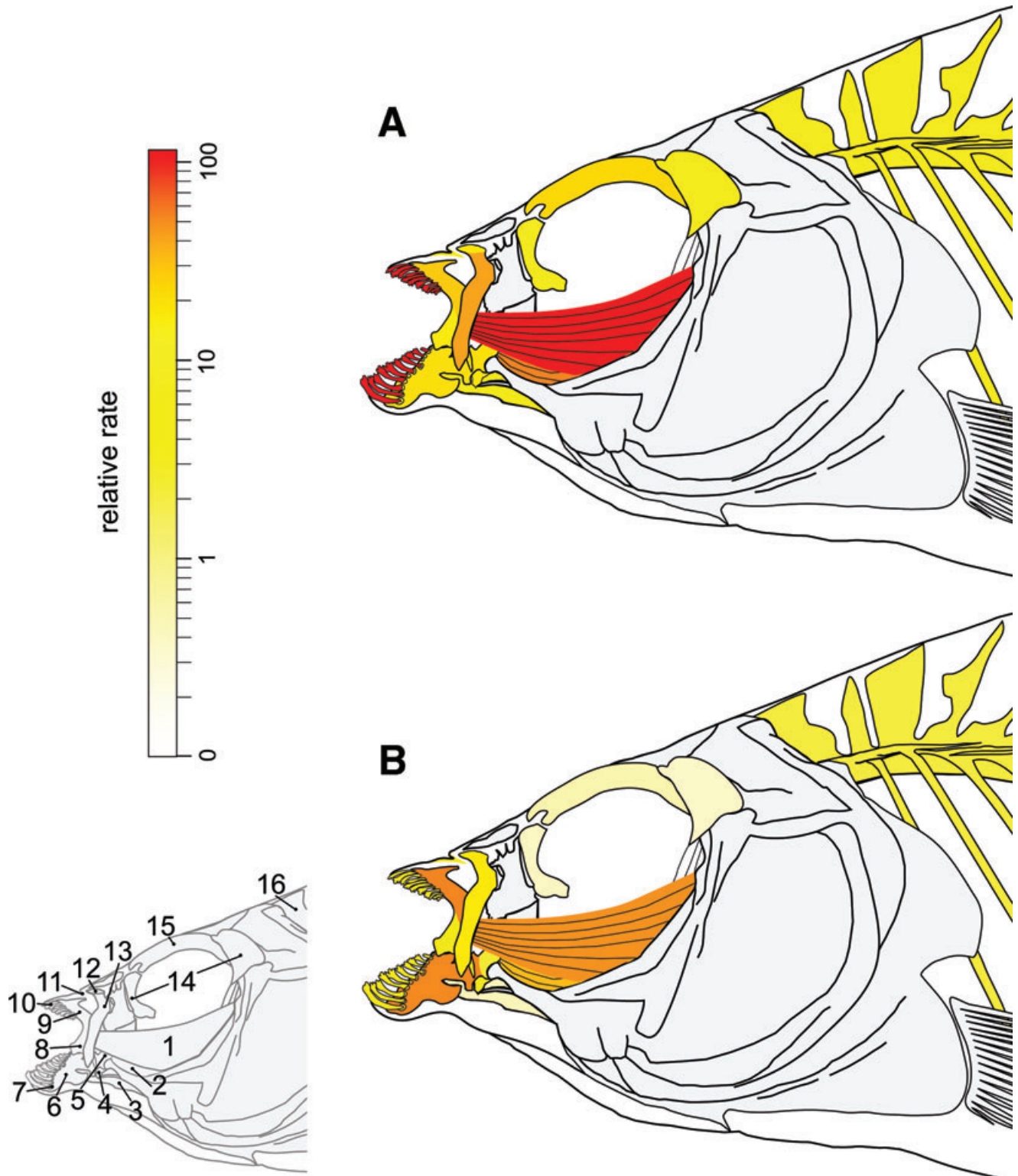
  

Trait	San Salvador rate	Background rate	Relative rate	$\Delta$ AIC <sub>c</sub>
1. A1	3.419 $\pm$ 1.128	0.074 $\pm$ 0.003	<b>47.479 <math>\pm</math> 15.693</b>	31.256 $\pm$ 2.571
2. A2 & A3	2.101 $\pm$ 0.675	0.061 $\pm$ 0.001	<b>34.459 <math>\pm</math> 11.224</b>	24.949 $\pm$ 2.290
3. Jaw length	28.349 $\pm$ 7.461	0.584 $\pm$ 0.013	<b>51.353 <math>\pm</math> 15.027</b>	36.065 $\pm$ 2.365
4. Opening lever	14.691 $\pm$ 4.975	1.949 $\pm$ 0.037	7.678 $\pm$ 2.657	4.148 $\pm$ 1.275
5. Closing lever	4.248 $\pm$ 1.397	1.630 $\pm$ 0.046	2.681 $\pm$ 0.948	-0.617 $\pm$ 0.741
6. Jaw protrusion	12.444 $\pm$ 2.661	1.709 $\pm$ 0.061	7.414 $\pm$ 1.526	5.534 $\pm$ 1.010
7. Gape	2.412 $\pm$ 0.766	5.105 $\pm$ 0.148	0.487 $\pm$ 0.153	0.210 $\pm$ 0.276
8. Maxilla	18.273 $\pm$ 6.210	1.041 $\pm$ 0.022	<b>17.154 <math>\pm</math> 5.632</b>	12.974 $\pm$ 1.776
9. Lower premaxilla	11.241 $\pm$ 3.824	2.006 $\pm$ 0.057	5.206 $\pm$ 1.415	2.323 $\pm$ 1.005
10. Upper premaxilla	29.094 $\pm$ 8.050	0.575 $\pm$ 0.012	<b>51.257 <math>\pm</math> 14.263</b>	36.245 $\pm$ 2.411
11. Maxillary process	23.055 $\pm$ 1.385	1.433 $\pm$ 0.077	<b>17.736 <math>\pm</math> 1.124</b>	20.328 $\pm$ 1.102
12. Number of teeth	31.791 $\pm$ 5.284	1.259 $\pm$ 0.027	<b>25.613 <math>\pm</math> 4.262</b>	25.458 $\pm$ 1.534
13. Tooth length	6.327 $\pm$ 1.784	1.367 $\pm$ 0.042	4.482 $\pm$ 0.982	1.889 $\pm$ 0.837
14. Orbit diameter	0.176 $\pm$ 0.009	0.628 $\pm$ 0.022	0.304 $\pm$ 0.017	-0.577 $\pm$ 0.116
15. Cranial width	0.507 $\pm$ 0.058	0.978 $\pm$ 0.022	0.537 $\pm$ 0.062	-1.441 $\pm$ 0.102
16. Body depth	2.211 $\pm$ 0.753	0.961 $\pm$ 0.034	2.241 $\pm$ 0.707	-0.616 $\pm$ 0.641

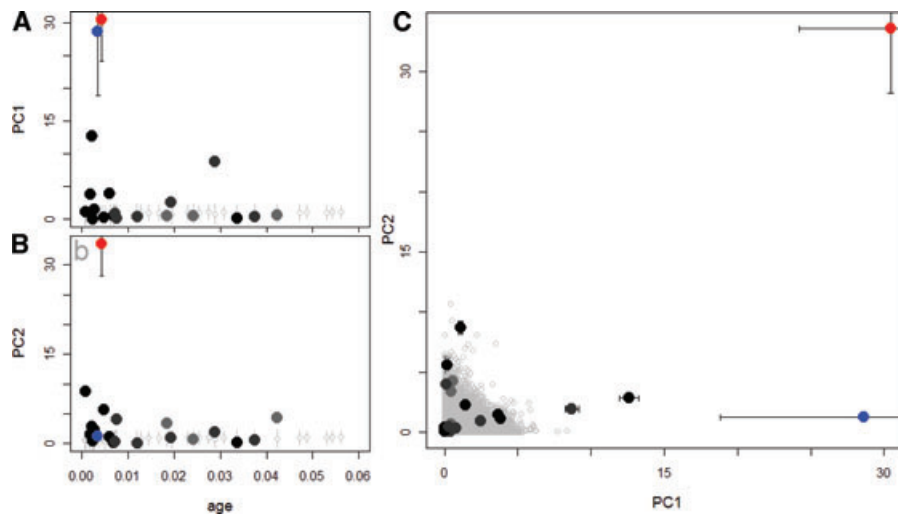
invasion of new, unoccupied habitats (e.g., Tobler and Carson 2010), yet two sympatric clades stand out from this adaptive milieu. Using ancestral reconstructions of diet, we estimated that the morphological diversification rate of trophic specialists in these two clades is up to 19 times faster than detritivore *Cyprinodon* species (Table 2). This suggests that the invasion of novel trophic niches in sympatry is playing a key role in generating rapid rates of morphological evolution.

#### TROPHIC NOVELTY AND ADAPTIVE RADIATION

*Cyprinodon* species within each sympatric clade have invaded a range of specialized trophic niches unique among *Cyprinodon*. Impressively, among all percomorphs, to our knowledge the most closely related scale-eaters to the scale-eating pupfish on San Salvador Island are the specialized cichlid species in the African Great Lakes (Fryer and Iles 1972) or the scale-eating mimic blenny (Sazima 1983). There are no known molluscivorous



**Figure 3.** *Cyprinodon* heat map with colors indicating the rates of morphological diversification in the (A) Lake Chichancanab and (B) San Salvador Island clades relative to all other Cyprinodontidae for 16 functional traits (see Table 1). Numbered diagram corresponds to numbered descriptions of the traits in Methods.



**Figure 4.** Relative rates of morphological diversification (mean  $\pm$  SE) on the (A) first and (B) second principal component axes relative to clade age. (C) The morphological diversification rate-space (mean  $\pm$  SE) on the first and second principal component axes. Observed rates are indicated for the Chichancanab (○) and San Salvador (●) clades, and all other two-species (●), three-species (●), and four-species (●) *Cyprinodon* clades measured; note that points are not statistically independent due to nested clade structure and shared histories. Means and standard errors were calculated by repeating rate analyses for 100 trees sampled from the stationary MCMC distribution. (A, B) Simulated relative rates of morphological diversification (mean  $\pm$  2SD) under a Brownian motion model for 1000 iterations are shown in gray (●). (C) Each simulation is indicated by one point (●) in the morphological diversification rate-space.

Cyprinodontidae or species with highly force-modified lower jaws similar to the ostracod and gastropod-eating specialist on San Salvador, although *C. pachycephalus* does include some snails in its diet, most likely due to their prevalence in its extreme environment (Minckley and Minckley 1986). There are piscivores and zooplanktivores within the *Orestias* radiation in Lake Titicaca (Parenti 1984) and a zooplanktivore *Aphanius* species (*A. asquamatus*), but no other *Cyprinodon* species have specialized on these niches.

Although ecological divergence is a widely recognized component of adaptive radiation, the invasion of trophic niches not

found among other members of a far more inclusive clade is a common and neglected feature of many classic adaptive radiations. For example, although beak diversity is also quite high in the Caribbean sister group to Darwin's finches (Burns et al. 2002), the specialized niches of blood-drinking, tool-using, and leaf-eating have only evolved on the Galapagos Islands (Grant and Grant 2007). Similarly, the three East African cichlid radiations contain the only known scale-eating, fry-stealing, parasite-feeding, and ambush piscivore cichlids (Fryer and Iles 1972; McKaye and Kocher 1983); *Drosophila* larvae specialize on parasitizing spider eggs only within the Hawaiian radiation in addition to

**Table 2.** Rates (mean  $\pm$  SE) of morphological diversification for trophic specialists (San Salvador clade:  $n = 2$ ; Chichancanab clade:  $n = 4$ ) and nonspecialist detritivore *Cyprinodon* (including all allopatric *Cyprinodon* plus the detritivore in each sympatric clade) on the first eight phylogenetically corrected principal component axes. Means and standard errors were calculated from 500 ancestral state reconstructions of diet (Bollback 2006) across 100 trees drawn from the stationary MCMC distribution. The difference in  $AIC_c$  scores is provided for the fit of the two-rate Brownian motion model relative to the one-rate model; negative scores indicate the one-rate model is favored. Relative rates for strongly supported two-rate models ( $\Delta AIC_c > 8$ ) are highlighted in bold.

PC axis	Trophic specialist rate	Nonspecialist rate	Relative rate	$\Delta AIC_c$
1	80.816 $\pm$ 8.400	10.985 $\pm$ 1.052	<b>19.145 <math>\pm</math> 2.159</b>	40.425 $\pm$ 1.689
2	17.529 $\pm$ 1.799	2.965 $\pm$ 0.237	<b>10.851 <math>\pm</math> 1.187</b>	24.663 $\pm$ 1.327
3	28.740 $\pm$ 3.469	5.637 $\pm$ 0.595	<b>12.440 <math>\pm</math> 1.795</b>	28.552 $\pm$ 1.484
4	10.612 $\pm$ 0.941	8.892 $\pm$ 0.649	1.920 $\pm$ 0.184	4.031 $\pm$ 0.472
5	15.064 $\pm$ 1.724	5.760 $\pm$ 0.708	<b>8.222 <math>\pm</math> 0.948</b>	18.831 $\pm$ 1.253
6	6.445 $\pm$ 0.468	2.078 $\pm$ 0.148	<b>4.450 <math>\pm</math> 0.272</b>	11.307 $\pm$ 0.908
7	16.344 $\pm$ 1.253	4.635 $\pm$ 0.531	<b>10.152 <math>\pm</math> 0.843</b>	29.088 $\pm$ 3.077
8	7.058 $\pm$ 0.499	6.592 $\pm$ 0.712	2.949 $\pm$ 0.202	7.745 $\pm$ 0.684

leaf-miners, stem, root, bark, flower, and tree sap specialists (Heed 1968; Carson 1971); and within an adaptive radiation of *Brocchinia* bromeliads on the Guianan table-top mountains are found the only carnivorous, myrmecophytous, mutualist with nitrogen-fixing bacteria, arboreal, and fire-resistant bromeliads among the Pitcairniodeae (Givnish et al. 1997). All these adaptive radiations show high levels of sympatry within isolated environments containing few competitors, facilitating the invasion of novel unoccupied fitness peaks. Although quantitative measures of relative speciation and morphological diversification rates are now used to define adaptive radiations (Losos and Miles 2002; Harmon et al. 2003; Rabosky and Lovette 2008), the presence of ecological novelty distinguishes many classic examples of adaptive radiation from their often morphologically diverse and speciose sister groups.

### CONTINGENCY AND DETERMINISM IN THE TWO SYMPATRIC CLADES

Both sympatric clades have evolved under remarkably similar conditions yet their rates of morphological diversification were uncorrelated, reflecting the unique trophic specialists in each. Both clades occur in large (10–15 km), shallow, saline lakes with only one other competing fish species, in both cases a species of *Gambusia* (Turner et al. 2008; Humphries and Miller 1981). An additional open-water fish species (*Atherinomorus stipes*) occurs in some San Salvador lakes, but this species is a nocturnal forager and does not appear to compete with the endemic *Cyprinodon* (C. H. Martin, unpubl. data). Moreover, the sister species to each sympatric clade are both wide-ranging coastal species with highly similar morphology (Fig. 2) and ecology, foraging primarily on algae and detritus (Miller et al. 2005). Because these sister species have a large range (Massachusetts to the Caribbean and the entire coastal Yucatan) and highly similar morphology across this range (Fig. 2), we can reasonably infer that the ancestral founding populations of each sympatric clade were also highly similar in morphology and ecology. Thus, not only have these sympatric radiations occurred in similar environments, they have also probably originated from morphologically and ecologically similar founding populations. Nonetheless, they have followed different trajectories in their evolution of specialist trophic niches. Chichancanab *Cyprinodon* diverged most rapidly in adductor muscle mass, tooth length, and maxilla length (Table 1, Figure 3), reflecting the large contrast between the piscivore and zooplanktivore. Zooplanktivores benefit from smaller mouth openings for generating greater acceleration and thus greater suction force on their prey (Holzman et al. 2008). Conversely, many piscivores use a larger component of ram and stronger jaws to capture large elusive prey at the expense of reduced suction force (Holzman et al. 2008). San Salvador *Cyprinodon* have diverged fastest in overall jaw size, including lower jaw and upper premaxilla length, reflect-

ing the trade-off between a mechanical advantage for crushing hard-shelled prey (shorter jaw, larger closing lever) versus rapid jaw opening and closing for quickly performing scale-removing strikes (longer jaw, shorter opening and closing levers). The scale-eaters have additionally retained a powerful closing bite, despite lower mechanical advantage, by increasing the proportional size of their adductor muscles.

The unique patterns of trophic specialization within *Cyprinodon* are in marked contrast to the numerous examples of parallel evolution of limnetic/benthic species pairs among temperate lake fish (Robinson and Wilson 1994; Schluter 2000) and other parallel patterns of divergence across the same taxa adapting to similar habitats (Losos 1998; Gillespie 2004; Langerhans and Dewitt 2004; Landry et al. 2007; Losos 2009). Although Lake Chichancanab might fit the limnetic/benthic/piscivore pattern seen in a few other sympatric radiations of fish (e.g., Gislason et al. 1999; Jonsson and Jonsson 2001; Schliewen et al. 2001), the San Salvador scale-eater clearly does not fit this mold. Differences in resource availability or fish communities between the two lakes might account for the observed specialization on different types of hard-shelled prey or the absence of zooplanktivory in San Salvador *Cyprinodon*, but it is harder to invoke these explanations for scale eating; fish communities in both lakes occur at high densities, comparable to the dense communities of rock-dwelling cichlids in Lake Malawi where scale-eating specialists have also evolved (see abundance data in Martin and Genner 2009), and scales are seemingly an omnipresent, renewable resource in all fish communities. Rather, it seems more likely that scale eating is an empty niche in Lake Chichancanab and intrinsic differences between the two founding lineages, such as in aggression or standing genetic variation for lower jaw size, are more likely to explain their different use of this niche.

### WHAT DRIVES TROPHIC INNOVATION?

We have argued for a strong link between trophic innovation and exceptional rates of morphological diversification, but it is not clear what causes trophic innovation. Across the entire range of *Cyprinodon* why do only two lakes contain sympatric radiations of trophic specialists? Ecological opportunity alone is not sufficient to explain this pattern because *Cyprinodon* populations have been repeatedly confined to isolated, extreme environments devoid of competitors. For example, in addition to those species confined to habitats with no other fish species present (Miller 1943; Deacon et al. 1995), there are also many isolated species coexisting with only a single species of *Gambusia* or another species of Poeciliid (Minckley and Minckley 1986; Miller et al. 2005), especially throughout the Caribbean (e.g., Rivas and Fink 1970; C. H. Martin, pers. obs.). Thus, it appears that the depauperate fish communities of *Cyprinodon* and *Gambusia* occurring in Lake Chichancanab and San Salvador's interior lakes

are not uncommon across the range of *Cyprinodon*. However, most of these isolated *Cyprinodon* populations are small relative to the large, high-density populations in Chichancanab and San Salvador, so large areas or population sizes may be necessary for the evolution of sympatric endemic sister species (e.g., Losos and Schluter 2000). A correlation between lake size and the number of endemic species is supported by the only other known sympatric radiation within Cyprinodontidae, the 24 endemic species of *Orestias* found in the 8300 km<sup>2</sup> Lake Titicaca (Parenti 1984). On the other hand, two distantly related Cyprinodontidae were both endemic to the 0.01 km<sup>2</sup> Potosí spring (Appendix S1) until this habitat was destroyed (Echelle et al. 1995). Finally, for almost every example of adaptive radiation, similar taxa, such as *Gambusia* in this case, have failed to radiate in the presence of the same ecological opportunity (Lovette et al. 2002; Seehausen 2006; Losos and Mahler 2010).

## CONCLUSION

Across the entire range of *Cyprinodon* only two isolated lakes contain sympatric radiations of species occupying unique trophic niches. This trophic innovation in sympatry is linked to extreme rates of morphological diversification along multiple independent axes, not clade age or the numerous invasions of extreme environments across this group. We propose that the invasion of novel ecological niches is a distinct mechanism driving the pattern of rapid species and morphological diversification observed in *Cyprinodon* and is shared with many classic examples of adaptive radiation. The ongoing process of speciation in both sympatric *Cyprinodon* radiations presents an opportunity to study the incipient evolution of novel ecological niches and the early stages of remarkable adaptive radiations.

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## Supporting Information

The following supporting information is available for this article:

**Table S1.** Mitochondrial gene regions, nucleotide substitution models, and partitioning schemes.

**Table S2.** Prior age distributions used to calibrate phylogenetic trees in BEAST analyses.

**Table S3.** Rates (mean  $\pm$  SE) of morphological diversification within two sympatric *Cyprinodon* clades from Lake Chichancanab, Mexico and San Salvador Island, Bahamas using time calibration priors on six nodes in our phylogenetic analysis.

**Table S4.** Loadings for the first eight principal component axes explaining 95% of the total morphological variation among the 16 traits measured.

**Appendix S1.** Species measured, location, source, and sample size in this study.

Supporting Information may be found in the online version of this article.

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