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DO FRESHWATER FISHES DIVERSIFY FASTER THAN MARINE FISHES? A TEST USING STATE-DEPENDENT DIVERSIFICATION ANALYSES AND MOLECULAR PHYLOGENETICS OF NEW WORLD SILVERSIDES (ATHERINOPSIDAE)

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Freshwater habitats make up only ∼0.01% of available aquatic habitat and yet harbor 40% of all fish species, whereas marine habitats comprise >99% of available aquatic habitat and have only 60% of fish species. One possible explanation for this pattern is that diversification rates are higher in freshwater habitats than in marine habitats. We investigated diversification in marine and freshwater lineages in the New World silverside fish clade Menidiinae (Teleostei, Atherinopsidae). Using a time-calibrated phylogeny and a state-dependent speciation–extinction framework, we determined the frequency and timing of habitat transitions in Menidiinae and tested for differences in diversification parameters between marine and freshwater lineages. We found that Menidiinae is an ancestrally marine lineage that independently colonized freshwater habitats four times followed by three reversals to the marine environment. Our state-dependent diversification analyses showed that freshwater lineages have higher speciation and extinction rates than marine lineages. Net diversification rates were higher (but not significant) in freshwater than marine environments. The marine lineage-through time (LTT) plot shows constant accumulation, suggesting that ecological limits to clade growth have not slowed diversification in marine lineages. Freshwater lineages exhibited an upturn near the recent in their LTT plot, which is consistent with our estimates of high background extinction rates. All sequence data are currently being archived on Genbank and phylogenetic trees archived on Treebase.

KEY WORDS: **Biogeography, BiSSE, extinction, macroevolution, species richness, speciation.**

Explaining the disparity in species richness among clades and areas is a major goal of evolutionary biology. One of the more intriguing patterns of biodiversity is the extraordinary number of species on continents compared to oceans (Vermeij and Grosberg 2010; Mora et al. 2011). In fact, it has been estimated that species diversity on continents is 25 times more than that found in oceans (Briggs 1994; Benton 2001), despite the fact that oceans are vastly greater in size. For aquatic organisms, freshwater (continental) habitats make up only ∼0.01% of available aquatic habitat and yet harbor 40% of all fish species, whereas marine habitats comprise >99% of available aquatic habitat and yield only 60% of fish diversity (Horn 1972; Lundberg et al. 2000;

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Leveque et al. 2008; Eschmeyer et al. 2010). The question of why continental diversity greatly exceeds than that found in oceans remains largely unanswered.

To explain the discrepancy between marine and freshwater diversity, several hypotheses emphasizing ecological factors (e.g., productivity, size of primary producers, and ecological specialization) have been proposed (May 1994; Vermeij and Grosberg 2010). Although ecological factors undoubtedly play a role in shaping biodiversity, ultimately disparity in species richness among clades and areas is the result of differences in net diversification (speciation minus extinction) and transition (or dispersal) rates between habitats (Barraclough and Nee 2001; Wiens and Donoghue 2004; Ricklefs 2007; Wiens et al. 2011), and the age of clades (McPeek and Brown 2007). Indeed, Benton (2001) hypothesized that the disparity in species richness among continental and oceanic lineages is an outcome of "rocketing diversification rates" in continental lineages compared to "the more sluggish rates of diversification of life in the sea." A largely unconsidered possibility is biased transition rates between marine and freshwater lineages over evolutionary time; there may be a high number of freshwater species because marine to freshwater transition rates are higher than freshwater to marine transitions (Maddison 2006; Maddison et al. 2007). Alternatively, freshwater lineages may be older than marine lineages, suggesting continents have simply had more time to accumulate diversity (McPeek and Brown 2007).

There are a number of reasons for predicting that freshwater lineages should have higher diversification rates than marine lineages. Causes of differential diversification may include environmental parameters, as well as intrinsic properties of organisms from each environment. Freshwater environments are generally expected to have more barriers limiting population connectivity, to have greater habitat complexity, and to be more heavily influenced by tectonic activity than marine environments (Strathmann 1990; May 1994). Marine organisms have greater geographic range size and less genetic structuring (Palumbi 1994; Bierne et al. 2003). All of these factors are known to influence speciation and extinction rates (Cracraft 1982; Jablonski 1987; Barraclough et al. 1998; Ribera et al. 2001; Jablonski 2008; Badgley 2010). However, the interaction of some of these parameters may yield unexpected patterns. For example, the probability of speciation should increase as geographic range size increases, and decrease as levels of gene flow increase (Kisel and Barraclough 2010; Kisel et al. 2011). So although oceans are vastly greater in size, thus predicting higher speciation rates, marine taxa also have higher levels of gene flow (Palumbi 1994; Bierne et al. 2003; Puebla 2009), which is thought to reduce the probability of speciation events (Kisel and Barraclough 2010). Benton (2001) also suggested that marine environments have strong ecological limits on clade growth, causing a slow-down in lineage accumulation, whereas continental environments have few ecological constraints. The first step toward disentangling how these various habitat parameters affect macroevolutionary patterns is testing the prediction that freshwater lineages have faster diversification rates.

Previous studies have demonstrated that macrohabitat (e.g., coral reefs vs. pelagic ocean) can influence diversification rates in marine fishes (Ruber et al. 2003; Ruber and Zardoya 2005; Alfaro et al. 2007). However, comparative studies on the diversification of marine and freshwater fishes have proven challenging. A recent study (Vega and Wiens 2012) addressed patterns of aquatic diversity but did not detect differences in diversification rates between marine and freshwater ray-finned fishes (Actinopterygii). However, due to the large number of actinopterygian species (~30,000), the authors were not able to use methods that explicitly estimate speciation and extinction rates for marine or freshwater character states (Maddison et al. 2007; FitzJohn et al. 2009; Vega and Wiens 2012). This makes it difficult to use clades that have mixed character states to infer diversification dynamics in a comparative framework (Maddison et al. 2007).

Testing for the effects of marine versus freshwater habitat on macroevolutionary patterns is possible using a time-calibrated phylogenetic framework that allows speciation, extinction, and character transition rates to be parameterized independently. By using a state-dependent model, we can explicitly estimate speciation and extinction rates as a function of a particular character state (Maddison et al. 2007). Ideally, the clade to be analyzed should contain multiple freshwater and marine lineages. However, many clades of fishes (and other taxa) are restricted to either marine or freshwater habitats (Lee and Bell 1999; Vermeij and Dudley 2000; Vermeij and Wesselingh 2002; Bloom and Lovejoy 2011). For this reason, New World silversides (Atherinopsidae) are an excellent system for investigating patterns of aquatic diversification because current taxonomy and (albeit limited) phylogenetic data suggests multiple marine/freshwater transitions have occurred. In this study, we focus on the subfamily Menidiinae, which is composed of 44 freshwater and 25 marine species. Marine species are distributed in the western Atlantic from southern Canada to northern Brazil, and in the eastern Pacific from Baja California to Peru. Marine silversides are generally near-shore fishes, with a few representatives in pelagic ocean habitats (Dyer 2006). Freshwater species are distributed in eastern United States and Canada, central and southern Mexico, and throughout Central America (Lee et al. 1980; Reis et al. 2003; Miller 2005; Dyer 2006). Freshwater silversides are largely riverine fishes, rarely occurring in natural lakes. The Central Mexican lakes clade of silversides is an exception with up to 13 species occurring in three lakes (Barbour 1973a,b; Bloom et al. 2009). Previous phylogenetic studies utilizing morphological (Chernoff 1986a; White 1986; Dyer and Chernoff 1996; Dyer 1998) and molecular data (Bloom et al. 2012) have supported the monophyly of Menidiinae. Although several

studies have investigated species level relationships in subclades within Menidiinae (Gosline 1948; Barbour 1973b; Echelle and Echelle 1984; Chernoff 1986b; Dyer 1998; Bloom et al. 2009), no molecular study has investigated the relationships among the major lineages of Menidiinae.

Here we use a phylogenetic approach to test for differences in diversification rates between marine and freshwater lineages of menidiine silversides. We generate a time-calibrated molecular phylogeny for Menidiinae, and reconstruct the number and timing of marine and freshwater habitat transitions. We ask whether freshwater lineages are older than marine lineages, and we use state-dependent models of diversification to test for differences in rates of speciation, extinction, and character state change between marine and freshwater lineages. Finally, we use lineage-through time (LTT) plots (Weir 2006; Rabosky and Lovette 2008a) to evaluate whether marine and freshwater lineages fit similar or different patterns of clade growth. Together, these tests and analyses provide novel insights into the macroevolutionary processes that determine differences in large-scale patterns of diversity between continents and oceans.

Materials and Methods

TAXON SAMPLING, DNA EXTRACTION, POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION, AND SEQUENCING

Our taxon sampling included 50 out of 74 species and representatives of all genera currently recognized in Menidiinae (Table S1). We also included, as outgroups, six species from Atherinopsinae, *Notocheirus hubbsi* (Notocheirinae), and *Atherinomorus stipes* (Atherinidae) for a total of 58 species in the dataset. Our taxon sampling includes representatives from every genus in the family Atherinopsidae except *Colpichthys* and comprises the most comprehensive molecular phylogenetic study of Atherinopsidae to date. Some data were available from previous studies (Bloom et al. 2009, 2012), whereas most were newly sequenced for this study; all new sequences were deposited in Genbank (Table 1).

Whole genomic DNA was extracted using DNeasy tissue kit (Qiagen, Valencia, CA). We PCR amplified fragments of two mitochondrial (nd2 and cytb) and two single-copy protein-coding nuclear (tmo4C4 and rag1) genes. For amplification and sequencing, we used protocols and primers from Bloom et al. (2009, 2012) for nd2, cytb, and rag1, and Lovejoy et al. (2004) for tmo4c4. Sequences were edited using the computer software Geneious v5.4 (Drummond et al. 2010) and aligned using the MUSCLE module (Edgar 2004) implemented in Genious. Following alignment, coding regions were translated to amino acids to confirm the integrity of reading frames and absence of stop codons. To test the monophyly of species and as a measure of quality control, we sequenced multiple individuals for nearly all newly sequenced species. We included multiple individuals of each species in the MrBayes analyses, but removed duplicate species representatives for all subsequent BEAST and diversification analyses described later.

PHYLOGENETIC ANALYSES AND DIVERSIFICATION TIME ESTIMATION

Best-fit models of nucleotide substitution were selected for each gene using Akaike information criteria in the program jModelTest (Posada 2008). We used BEAST v1.6.1 (Drummond and Rambaut 2007) to jointly estimate phylogeny and divergence times under a relaxed clock, uncorrelated lognormal model (Drummond et al. 2006) that allows rates to vary among branches. The dataset was partitioned by gene, with each partition unlinked and set to a general time reversible (GTR)ur model with γ-distributed rate heterogeneity. We used a birth-death prior for rates of cladogenesis and ran two independent analyses of 100 million generations sampling every 1000 generations. We used Tracer 1.5 (Drummond and Rambaut 2007) to evaluate convergence and mixing of runs and to verify that effective sample sizes were >200 for all parameters. We determined that the first 30 million generations from the Markov chain Monte Carlo (MCMC) sample were a conservative burn-in. The two converged runs were combined using LogCombiner v1.6.1 (Drummond and Rambaut 2007) and the maximum credibility tree (MC tree) was generated in TreeAnnotator v1.6.1 (Drummond and Rambaut 2007).

We used three fossil constraints in the BEAST analysis. A fossil *Basilichthys* is dated to the late Miocene (Rubilar 1994) and was used to constrain the *Basilichthys/Odontesthes* clade to a hard minimum bound of 5.33 ma with a soft upper bound of 75 ma based on previous molecular estimates that show Atherinomorpha is less that 75 million years old (Alfaro et al. 2009; Santini et al. 2009). A fossil *Menidia* from Oklahoma was dated to the lower Pliocene (Hubbs 1942). This fossil is a crown *Menidia* and was used to constrain the clade including *Menidia beryllina*, *Menidia colei*, and *Menidia peninsulae* with a hard minimum bound of 1.8 ma and a soft upper bound of 75 ma. A fossil *Chirostoma* is known from the Lerma Basin in Central Mexico and dated to the Pleistocene with a minimum age reported of 46,000 years before present (Bradbury 1971; Piller and Barbour, in press). The fossil *Chirostoma* is a member of the crown "humboldtianum" clade of large-bodied *Chirostoma* (Bradbury 1971; Echelle and Echelle 1984; Bloom et al. 2009). This fossil was used to date the clade including *C. humboldtianum*, *C. sphryaena*, *C. lucius*, *C. chapalae*, *C. estor*, *C. grandocule*, and *C. consocium* to a hard minimum age of 0.0046 ma and a soft upper bound of 5.33 ma based on the fossil *Menidia* (Hubbs 1942). We used exponential priors for all fossil constraints because this distribution is appropriate for crown fossils and when no information is available to determine the shape of a lognormal distribution (Ho and Phillips 2009).

Table 1. Summary of specimens used in this study, including habitat (M = marine, F = Freshwater) and associated Genbank and museum numbers.

Genus	Species	Habitat	CytB	ND2	TMO	RAG1	Museum number	Collection locality
Atherinella	balsanas	F	KC736414	KC736421	KC633404	KC669427	SLU 6637	Rio Cancita, Michoacan, Mexico
Atherinella	balsanas	F	KC736415	KC736422	KC633405	KC669428	SLU 6637	Rio Cancita, Michoacan, Mexico
Atherinella	brasiliensis	M	KC736412	KC736425	KC633402	KC669431	ROM 88861	Chatham, Trinidad
Atherinella	brasiliensis	M	KC736413	KC736426	KC633403	KC669432	ROM 88861	Chatham, Trinidad
Atherinella	chagresi	F	KC736363	KC736429	KC633337	KC669435	STRI-02090	Rio Canazas, Panama
Atherinella	alvarezi	F	KC736374	KC736416	KC633347	KC669424	SLU 6772	Laguna de Caobas, Mexico
Atherinella	alvarezi	F	KC736375	KC736417	KC633348	na	SLU 6772	Laguna de Caobas, Mexico
Atherinella	ammophila	F	KC736391	KC736418	KC633366	KC669425	SLU-TC 380	Rio la Palma, Mexico
Atherinella	argentea	M	JQ282017	KC736419	KC633335	JQ282062	ROM 91571	Playla Cruzas, Panama
Atherinella	argentea	M	KC736360	KC736420	KC633336	KC669426	ROM 91571	Playla Cruzas, Panama
Atherinella	blackburni	M	KC736356	KC736423	KC633333	KC669429	ROM 93997	Playa Baraka, Galeta, Panama
Atherinella	blackburni	M	KC736357	KC736424	KC633334	KC669430	ROM 93997	Playa Baraka, Galeta, Panama
Atherinella	crystallina	F	KC736346	KC736427	KC633321	KC669433	SLU 6087	Rio Acaponeta, Nayarit, Mexico
Atherinella	crystallina	F	KC736347	KC736428	KC633322	KC669434	SLU 6087	Rio Acaponeta, Nayarit, Mexico
Atherinella	pellosemion	F	KC736348	KC736432	KC633323	KC669438	SLU 6115	Rio Mancuernas, Nayarit, Mexico
Atherinella	pellosemion	F	KC736349	KC736433	KC633324	KC669439	SLU 6115	Rio Mancuernas, Nayarit, Mexico
Atherinella	colombiensis	F	KC736364	KC736430	KC633338	KC669436	STRI 02097	Rio San Juan, Colombia
Atherinella	colombiensis	F	KC736365	KC736431	KC633339	KC669437	STRI 02097	Rio San Juan, Colombia
Atherinella	guatamalanensis	F	KC736380	EF602045	KC633353	KC669443	SLU 5012	Rio Tehuantepec, Mexico
Atherinella	guatamalanensis	F	KC736381	KC736434	KC633354	KC669444	SLU 5085	Laguna Coyucan, Mexico
Atherinella	guatamalanensis	F	KC736386	KC736435	KC633360	KC669445	SLU 6074	El Teucan Lagoon, Mexico
Atherinella	guatamalanensis	F	KC736387	KC736436	KC633361	KC669446	SLU 6074	El Teucan Lagoon, Mexico
Atherinella	hubbsi	F	KC736388	KC736437	KC633362	KC669447	SLU 6853	Costa Rica
Atherinella	hubbsi	F	JQ282020	KC736438	KC633363	JQ282065	SLU 6853	Costa Rica
Atherinella	marvalae	F	JQ282021	KC736439	KC633355	JQ282066	SLU 6690	Rio Papaloapan, Mexico

Continued.

Table 1. Continued.

Genus	Species	Habitat	CytB	ND2	TMO	RAG1	Museum number	Collection locality
Atherinella	marvalae	F	KC736382	KC736440	KC633356	KC669448	SLU 6690	Rio Papaloapan, Mexico
Atherinella	milleri	F	KC736378	KC736441	KC633351	KC669449	SLU 5104	Cangrejal river, Honduras
Atherinella	milleri	F	KC736379	KC736442	KC633352	KC669450	SLU 5104	Cangrejal river, Honduras
Atherinella	serrivomer	M	KC736358	KC736450	na	KC669458	ROM 93998	Play Peten, Boca Parita, Panama
Atherinella	serrivomer	M	KC736359	KC736451	na	KC669459	ROM 93998	Play Peten, Boca Parita, Panama
Atherinella	panamensis	M	KC736361	KC736443	na	KC669451	ROM 93999	Playa la Cruzas, Panama
Atherinella	panamensis	M	KC736362	KC736444	na	KC669452	ROM 93999	Playa la Cruzas, Panama
Atherinella	sallei	F	KC736383	KC736445	KC633357	KC669453	SLU 5005	Rio Hueyapan, Mexico
Atherinella	sallei	F	KC736384	KC736446	KC633358	KC669454	SLU 5005	Rio Hueyapan, Mexico
Atherinella	sardina	F	KC736389	KC736447	KC633364	KC669455	ROM 94000	L. Apoyo, Nicaragua
Atherinella	sardina	F	KC736390	KC736448	KC633365	KC669456	ROM 94000	L. Apoyo, Nicaragua
Atherinella	schultzi	F	KC736376	EF602044	KC633349	na	SLU 5103	Rio Palenque, Mexico
Atherinella	schultzi	F	KC736377	KC736449	KC633350	KC669457	SLU 5103	Rio Palenque, Mexico
Atherinella	n. sp.	M	KC736350	KC736453	KC633327	KC669460	SLU 6853	Pacific Ocean, Mazatlan, Mexico
Atherinella	n. sp.	M	KC736351	KC736454	KC633328	KC669442	SLU 6853	Pacific Ocean, Mazatlan, Mexico
Atherinella	schultzi	F	KC736385	KC736452	KC633359	KC669461	SLU TC5008	Rio Almoloya, Mexico
Atherinella	starksi	M	KC736352	KC736455	KC633329	KC669462	ROM 94001	Tabago Island, Panama
Atherinella	starksi	M	KC736353	KC736456	KC633330	KC669463	ROM 94001	Tabago Island, Panama
Atherinomorus	stipes	M	JQ282023	KC736457	KC633315	JQ282068	ROM 91573	Barbados
Atherinomorus	stipes	M	KC736343	KC736458	KC633316	KC669464	ROM 91573	Barbados
Atherinops	affinis	M	na	KC736459	KC633397	JQ282061	SIO 0581	Pacific Ocean, California, USA
Atherinopsis	californiensis	M	JQ282018	KC736460	KC633398	JQ282063	SIO 03458	Pacific Ocean, California, USA
Basilichthys	semotilus	F	JQ282024	EF602042	na	JQ282069	ANSP 180736	R. Santuario, Peru
Chirostoma	arge	F	na	EF602099	KC633389	na	SLU 5110	Laguna Negritas, Mexico
Chirostoma	attenuatum	F	KC736404	EF602083	KC633387	KC669465	SLU 5036	L. Zirahuen, Mexico

Continued.

Table 1. Continued.

Genus	Species	Habitat	CytB	ND2	TMO	RAG1	Museum number	Collection locality
Chirostoma	attenuatum	F	KC736405	EF602082	KC633388	KC669466	SLU 5036	L. Patzcuaro, Mexico
Chirostoma	chapalae	F	KC736397	EF602075	KC633375	KC669467	SLU 5016	L. Chapala, Mexico
Chirostoma	consocium	F	JQ282025	EF602078	KC633381	JQ282070	SLU 5023	L. Chapala, Mexico
Chirostoma	consocium	F	KC736401	KC736461	KC633382	KC669468	SLU 5023	L. Chapala, Mexico
Chirostoma	contrerasi	F	KC736406	EF602098	KC633390	KC669469	SLU 5080	Rio Laja, Mexico
Chirostoma	estor	F	KC736403	EF602068	KC633385	KC669470	SLU 5114	L. Patzcuaro, Mexico
Chirostoma	grandocule	F	KC736369	EF602061	na	na	SLU 5118	L. Patzcuaro, Mexico
Chirostoma	humboldtianum	F	KC736402	EF602070	KC633383	na	SLU 5095	San Pedro Lagunillas, Mexico
Chirostoma	humboldtianum	F	JQ282026	EF602071	KC633384	JQ282071	SLU 5011	Lago de Zacapu, Mexico
Chirostoma	jordani	F	KC736407	EF602086	KC633391	KC669471	SLU 5033	L. Chapala, Mexico
Chirostoma	jordani	F	JQ282027	EF602090	KC633392	JQ282072	SLU 5033	L. Cuitzeo, Mexico
Chirostoma	labarcae	F	KC736399	EF602084	KC633378	KC669472	SLU 5017	L. Chapala, Mexico
Chirostoma	labarcae	F	JQ282073	EF602085	KC633379	JQ282028	SLU 5017	L. Chapala, Mexico
Chirostoma	lucius	F		EF602059		na	SLU 5022	L. Negritos, Mexico
Chirostoma	patzcuaro	F	JQ282029	EF602063	KC633386	JQ282074	SLU 5117	L. Patzcuaro, Mexico
Chirostoma	promelas	F	KC736368	EF602060	KC633342	na	SLU-TC 925	Tizaplan Hatchery, Mexico
Chirostoma	riojai	F	KC736398	EF602096	KC633376	KC669473	SLU 5079	L. Guadalupe Victoria, Mexico
Chirostoma	riojai	F	JQ282030	EF602097	KC633377	JQ282075	SLU 5079	L. Guadalupe Victoria, Mexico
Chirostoma	sphyraena	F	KC736400	EF602065	KC633380	KC669474	SLU 5025	L. Chapala, Mexico
Labidesthes	vanhyningi	F	KC736409	EF602057	KC633395	KC669475	SLU 5106	Pine Log Creek, FL, USA
Labidesthes	sicculus	F	JQ282031	KC736462	KC633396	JQ282077	SLU-TC 607	Duck River, TN, USA
Leuresthes	tenuis	M	JQ282032	KC736463	KC633399	JQ282078	SIO 0563	Pacific Ocean, California, USA
Melanorhinus	microps	M	KC736344	KC736474	KC633317	KC669482	ROM 91572	Archers Bay, Barbados
Melanorhinus	microps	M	JQ282037	KC736475	KC633318	JQ282083	ROM 91572	Archers Bay, Barbados

Continued.

Table 1. Continued.

Genus	Species	Habitat	CytB	ND2	TMO	RAG1	Museum number	Collection locality
Membras	gilberti	M	JQ282034	KC736469	KC633331	JQ282080	ROM 91569	Tabago Island, Panama
Membras	gilberti	M	KC736355	KC736470	KC633332	KC669479	ROM 91569	Tabago Island, Panama
Membras	martinica	M	JQ282035	KC736471	KC633369	KC669480	SLU 5102	Wrightsville Beach, NC, USA
Membras	martinica	M	KC736393	KC736472	KC633370	JQ282081	SLU 5102	Wrightsville Beach, NC, USA
Menidia	beryllina	F	KC736408	EF602049	KC633393	KC669476	SLU 5108	Bayou Lacombe, LA, USA
Menidia	beryllina	F	JQ282033	KC736464	KC633394	JQ282079	ROM 91570	L. Ponchartrain, LA, USA
Menidia	colei	M	KC736372	KC736465	KC633345	KC669477	SLU-TC 1914	Laguna de Caobas, Mexico
Menidia	colei	M	KC736373	KC736466	KC633346	KC669478	SLU-TC 1915	Laguna de Caobas, Mexico
Menidia	extensa	F	KC736370	KC736467	KC633343	na	na	Lake Waccamaw, NC, USA
Menidia	extensa	F	KC736371	KC736468	KC633344	na	na	Lake Waccamaw, NC, USA
Menidia	menidia	M	JQ282036	KC736473	KC633367	JQ282082	SLU-TC 2240	Wrightsville Beach, NC, USA
Menidia	menidia	M	KC736392	EF602050	KC633368	KC669481	SLU-TC 2241	Wrightsville Beach, NC, USA
Menidia	peninsulae	M	JQ282038	KC736476	KC633319	JQ282084	SLU 5107	Panama City, FL, USA
Menidia	peninsulae	M	KC736345	KC736477	KC633320	KC669483	SLU 5107	Panama City, FL, USA
Notocheirus	hubbsi	M	JQ282012	na	na	JQ282054	na	Atlantic Ocean, Argentina
Odonthesthes	mauleanum	F	KC736410	KC736478	KC633400	KC669485	na	Rio Itata, Chile
Odonthesthes	smitti	M	KC736411	KC736479	KC633401	KC669486	na	Puerto Madryn, Argentina
Poblana	letholepis	F	KC736367	EF602105	KC633341	KC669490	SLU 5116	L. Preciosa, Mexico
Poblana	squamata	F	KC736366	EF602112	KC633340	na	SLU 5115	L. Preciosa, Mexico
Poblana	alchichica	F	KC736395	EF602109	KC633373	KC669487	SLU 5034	L. Alchichica, Mexico
Poblana	alchichica	F	KC736396	EF602110	KC633374	KC669488	SLU 5034	L. Alchichica, Mexico
Poblana	ferdebueni	F	KC736394	EF602100	KC633371	KC669489	SLU 5028	L. Chignahuapan, Mexico
Poblana	ferdebueni	F	JQ282039	EF602101	KC633372	JQ282085	SLU 5028	L. Chignahuapan, Mexico

We estimated phylogenetic relationships using a mixed-model partitioned-by-gene Bayesian analysis implemented in MrBayes v3.1.2 software (Ronquist and Huelsenbeck 2003). We ran two MrBayes analyses, which consisted of four independent chains run for 10 million generations, sampling every 1000 generations with all parameters unlinked and default priors. Convergence of MrBayes analyses was assessed by comparing likelihood states over generations using the sump command in MrBayes and by confirming that standard deviation of split frequencies remained below 0.01 and potential scale reduction factors were 1.0. Adequate mixing of chains was confirmed by determining that acceptance rates were between 10% and 70%. MrBayes tree searches were used to confirm the topology recovered in our BEAST analyses, but subsequent diversification analyses and trait reconstructions were estimated using the trees from the BEAST analyses.

LINEAGE DIVERSIFICATION ANALYSES

Our diversification analyses were conducted on Menidiinae only by pruning all non-Menidiinae from the tree. We used a state-dependent speciation and extinction model to estimate diversification rates for lineages with marine or freshwater states. For binary characters, the BiSSE model estimates the probability that a lineage evolved as observed given a set of speciation (λ), extinction (μ), and character transition (q01 and q10) parameters (Maddison et al. 2007). Using this framework, we compared the fit of models with unconstrained parameters (speciation, extinction, and transition rates allowed to vary) to models with these parameters constrained to be equal to explicitly test the hypothesis that speciation and extinction rates were different between marine and freshwater habitats, and that there were asymmetrical transition rates between habitats (i.e., test for asymmetrical character transitions). Recent implementations of BiSSE correct for a known proportion of missing species in each character state. We accounted for missing species using the proportions of included and missing taxa for each character state: 13 marine (proportion = 0.5), 11 freshwater (proportion = 0.2292), assuming missing species represent a random sample. The BiSSE models we implemented assume that parameters are constant through time. Although more complex models in which parameters change as a function of time can also be used, these greatly increase the number of estimated parameters. Given the size of our tree (50 species) we felt it was unwise to test models with time variable parameters.

Marine lineages might transition to a freshwater state (and vise versa) via an intermediate state defined as occupancy of both freshwater and marine habitats. Indeed, two silverside lineages that are primarily freshwater are known to occasionally occur estuarine habitats (*M. beryllina* and *A. guatamalanensis*). We coded these species as transitional and used GeoSSE (Goldberg et al. 2011), which allows for transitional states, as an alternative to BiSSE.

Model parameters of our BiSSE analysis were optimized in a maximum likelihood framework on our MC tree and best-fit models were selected using Akaike Information Criteria (AIC) following Maddison et al. (2007). We also estimated parameters on our MC tree in a Bayesian framework using MCMC sampling to more effectively search for optimal parameter estimates. Our MCMC parameter searches consisted of 10,000 iterations with 2500 discarded as burn-in. We used maximum likelihood parameter estimates as starting values in the MCMC analyses. Exponential priors were used following the examples in the DiversiTree package (FitzJohn et al. 2009). To incorporate branch length and topology uncertainty, we obtained parameter estimates across a sample of 97 of our Bayesian trees generated in BEAST. Trees were sampled every 700,000 generations following the burn-in period. For each tree, we performed MCMC parameter searches of 5000 iterations, with a 2000 iteration burn-in. All BiSSE analyses were conducted in the R package DiversiTree (FitzJohn et al. 2009).

Our silverside tree has only 50 species, yet our best-fit model using BiSSE has five parameters (Table 2). We ran a power analysis to determine if our best-fit model is appropriate and if our data provided sufficient signal to obtain reasonable parameter estimates. Using our maximum likelihood parameter estimates, we simulated 1700 trees and their associated character state data (marine or freshwater) under the best-fit model (CRλ01, CRμ01, CRq). Each tree was simulated with 74 tips (the total number of species in our group), and 24 tips were randomly pruned, resulting in trees with 50 tips (the actual level of sampling we obtained). Maximum likelihood was then used to fit all the models in Table 2 to each simulated tree, and log-likelihoods and parameter estimates were recorded. If the parameter estimates we obtained under our best-fit model are reasonable, then these parameter estimates should be similar to those from simulated data. Similarly, if the best-fit model represents a reasonable model for our data, then it should also be chosen as the best-fit model in simulated datasets. We used AIC and likelihood ratio tests to determine model fit. For the likelihood ratio test, twice the difference in log-likelihoods between constrained and unconstrained models should follow a χ^2 distribution with degrees of freedom equal to the difference in the number of free parameters between the two models. To test this assumption, we obtained empirical estimates of the critical cutoff value using simulation (see supporting online methods). The empirical cutoff value (3.43) was similar to the expected cutoff value (3.84) under a χ^2 distribution with 1 degree of freedom. Given the similarity, we consider the χ^2 approximation appropriate for significance testing with likelihood ratio tests (see S1).

Table 2. Summary of model statistics from BiSSE analyses of speciation, extinction, and habitat transitions of silversides. Marine habitats are coded as state 0 and freshwater habitats as state 1. Speciation rates are λ, extinction rates are μ, and character transition rates are q. An "=" sign indicates model parameters constrained as equal, and a "≠"sign indicates parameters estimated independently for each habitat state. The best-fit model is in bold.

Model	Ln *L*	Parameters	AIC	ΔAIC	exp(−ΔAIC/2)	Akaike weights
λ0 = λ1, q01 = q10	−160.6444	2	325.2888	27.61	1.01299E−06	0.0000
λ0 = λ1, μ0 = μ1, q01 = q10	−153.2400	3	312.4800	14.80	0.000612354	0.0004
λ0 ≠ λ1, q01 = q10	−153.1721	3	312.3442	14.66	0.000655377	0.0005
λ0 ≠ λ1, q01 ≠ q10	−152.4243	4	312.8486	15.17	0.000509286	0.0004
λ0 ≠ λ1, μ0 = μ1, q01 = q10	−152.2363	4	312.4726	14.79	0.000614624	0.0004
λ0 ≠ λ1, μ0 ≠ μ1, q01 = q10	**−143.8418**	**5**	**297.6836**	**0.00**	**1**	**0.7294**
λ0 ≠ λ1, μ0 ≠ μ1, q01 ≠ q10	−143.8401	6	299.6802	2.00	0.368505368	0.2688

ANCESTRAL CHARACTER RECONSTRUCTIONS AND LINEAGE DIVERSIFICATION THROUGH TIME ANALYSIS

We coded marine and freshwater habitats as discrete, unordered character states. All character reconstructions were conducted on the chronogram resulting from the BEAST analyses. We used maximum parsimony (MP) and maximum likelihood (ML) in Mesquite v2.6 (Maddison and Maddison 2011) to reconstruct ancestral character states and determine the number of transitions between marine and freshwater habitats. Maximum likelihood reconstructions were estimated using the Mk model (Pagel 1999).

We generated LTT plots to evaluate patterns of clade growth. The log number of LTT indicates the relative rate of lineage accumulation (Weir 2006). If there are no limits to clade growth, lineages are expected to accrue exponentially through time yielding an LTT plot with a straight slope. A slow-down in lineage accumulation over time is characterized by a decline in slope through time and indicates the existence of a diversity-dependent threshold on clade growth. A strong upturn in the slope of LTT plots near the recent is the signature of high background extinction (although a recent increase in speciation rate can mirror this pattern; Weir 2006; Rabosky and Lovette 2008a). We explored patterns of clade growth for the entire Menidiinae clade as well as clade growth separately in marine and freshwater habitats. We followed the approach of Weir (2006) to generate LTT plots for marine and freshwater clades separately; this method uses information from ancestral state reconstructions to determine lineage accumulation patterns based on character state (assuming transitions occur at nodes, nodes representing a transition to, or diversification within the marine state were included in the marine LTT plot; nodes representing transition to, or diversification within a freshwater state were included in the freshwater LTT plot; see Weir 2006 for details). We also calculated the γ statistic (Pybus and Harvey 2000) for marine and freshwater lineages independently (using the same separation of nodes used done in the LTT analysis) using the R package Laser (Rabosky 2006b).

Results

MOLECULAR DATA AND PHYLOGENETICS

Our final dataset consisted of 1047bp from nd2, 1121bp of cytb, 534bp of tmo4c4, and 1141bp of rag1. The combined dataset included 4143 characters, 1441 of which were parsimony informative. Figure 1 shows our time-calibrated phylogeny, which is well resolved and has strong support for clades associated with habitat transitions, indicating phylogenetic uncertainty is not problematic for character reconstructions (Figs. S1 and S2). We find strong support for a monophyletic Atherinopsidae, and our focal clade, Menidiinae. Within Menidiinae, we found two major species groups: the Menidiini, distributed in North America, the Caribbean, and the Central Mexican Plateau, and the Membradini, distributed in Central America and southern Mexico. Our analyses place *Atherinella brasiliensis* as sister to all other members of Menidiinae; previously this taxon was thought to be a member of the Membradini clade (Chernoff 1986a). We also recovered *Melanorhinus microps* as sister to all other Menidinii, rather than in its previously proposed position as a member of Membradini (Chernoff 1986a; Dyer 1997). Many currently recognized genera were not recovered as monophyletic and are likely in need of taxonomic revision. Aside from these differences, our results are consistent with previous studies on silverside phylogenetics at or above the genus level (Chernoff 1986a; Dyer and Chernoff 1996; Dyer 1998, 2006; Bloom et al. 2009; Bloom et al. 2012).

ANCESTRAL CHARACTER RECONSTRUCTIONS

Ancestral character reconstructions indicate a marine state for the most recent common ancestor (MRCA) of Menidiinae and both of the major clades Menidiini and Membradini. Within Menidiinae, there were four independent transitions from marine to freshwater habitats, occurring in the following geographic regions: (1) Mississippi and Atlantic drainages of eastern North America and the central plateau region of Mexico (within Menidiini); (2) Atlantic drainages along the Isthmus of Tehuantepec and western

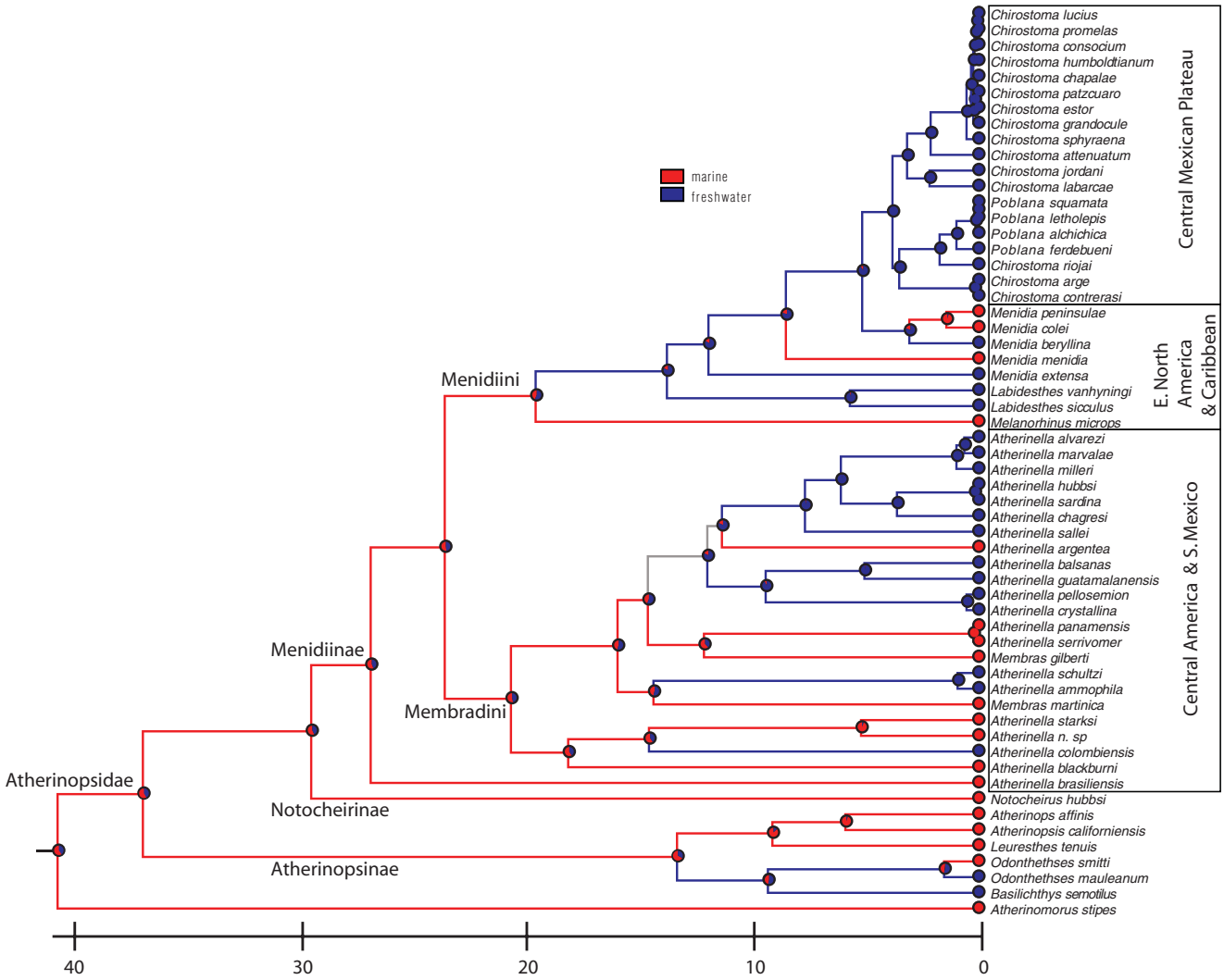

Figure 1. **Time-calibrated maximum clade credibility phylogeny of silversides from BEAST analysis of four-gene dataset (referred to in the text as MC tree). Branch colors and circles at nodes indicate maximum likelihood ancestral character reconstructions for marine (red) and freshwater (blue) habitats. Branches are proportional to absolute time and the x-axis is in millions of years (ma) before present day.**

margins of the Yucutan Peninsula in southern Mexico (*Atherinella schultzi* and *A. ammophila*); (3) coastal and southern Mexico and Central America, including Atlantic and Pacific drainages (clade including 11 species of *Atherinella*); and (4) the Pacific slope of Colombia (*Atherinella colombiensis*). There were also three reversals from freshwater back to marine habitats. These freshwater to marine transitions occurred: (1) along the Atlantic coast of North America (*Menidia menidia*), (2) in the Gulf of Mexico (*Menidia peninsulae* and *Menidia colei*), and (3) along the Atlantic coast of Central America (*Atherinella argentea*). Qualitatively, this suggests that asymmetrical transition rates do not explain freshwater species richness; quantitative evidence is provided later.

DIVERSIFICATION TIMES

Our time-calibrated phylogeny is deposited on TreeBase 13962. Our diversification time analysis dates the MRCA of Atherinopsidae to 36.94 ma, and the MRCA of Menidiinae to 26.89 ma (Figs. 1 and S2). The oldest node reconstructed as freshwater was the MRCA of *Melanorhinus* and members of Menidiini, dated to 19.59 ma. Within the Membradini clade, the oldest reconstructed freshwater node was dated to 14.59 ma. Over half of the 37 freshwater species included in our study date to less than 3.5 ma, and nine are members of a clade from the central Mexican plateau region that date to only 0.52 ma. Together this indicates that marine Menidiinae are at least 7 million years older than the earliest freshwater lineage and the majority of freshwater silverside lineages are relatively young.

SPECIATION, EXTINCTION, AND TRANSITION RATES

Our BiSSE analyses showed that pure birth models without extinction were poorly supported (Akaike weight < 0.0006; Table 2). Similarly, models constraining speciation and/or extinction rates

Table 3. Speciation rate, extinction rate, and transition rate estimates from the fully unconstrained model ($\lambda 0 \neq \lambda 1$, $\mu 0 \neq \mu 1$, $q01 \neq q10$) in the BiSSE analyses. See Table 2 for symbol definitions.

$\lambda 0$	$\lambda 1$	$\mu 0$	$\mu 1$	$q01$	$q10$
0.05684038	0.81213433	0.01771647	0.76005844	0.01835187	0.02166593

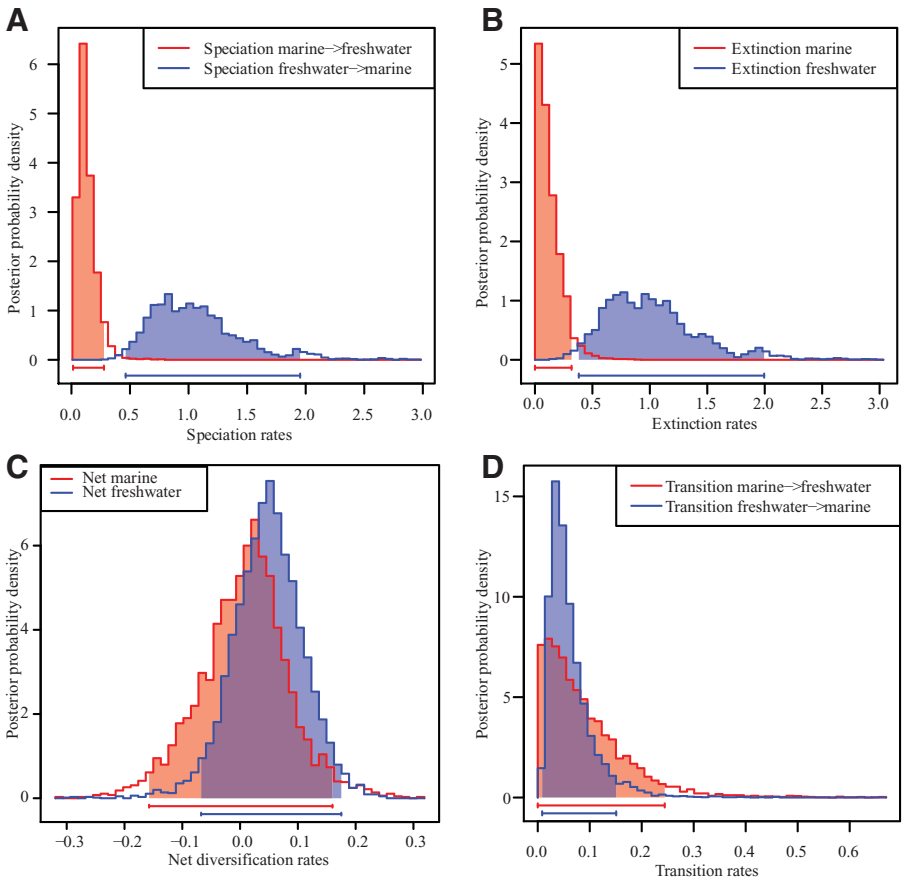

Figure 2. Posterior distribution of speciation rates (A), extinction rates (B), net diversification rates (C), and character transition rates (D) for marine (red) and freshwater (blue) silverside lineages from our BiSSE Bayesian analysis.

to be equal for marine and freshwater species provided a much worse fit (Akaike weights < 0.0004; Table 2) than models that allowed these rates to vary. Our best-fit model was one in which speciation and extinction rates differed greatly between marine and freshwater biomes, but character transition rates were symmetrical (Akaike weight = 0.73; Tables 2). The next best-fit model was the full BiSSE model in which all parameters were asymmetrical (Akaike weight = 0.27; Table 2 and 3).

We explored the posterior distribution of parameter values along our MC tree under the full BiSSE model (Fig. 2). These distributions show that speciation and extinction rates were much greater in freshwater than marine lineages. For speciation and extinction rates, the 95% credible intervals did not overlap between freshwater and marine lineages (indicating a high degree of certainty in these estimates), but overlap did occur for net diversification rates and transitions rates between marine and freshwater.

To determine whether model parameters varied significantly between marine and freshwater while simultaneously accounting for topology and branch length uncertainty, we estimated posterior distributions of parameter values across a sample of 97 posterior trees for the full model. For each Bayesian MCMC sample we subtracted parameter estimates in marine environments from those in freshwater. If rates are significantly different between marine and freshwater, the resulting posterior distributions will not overlap in their 95% credible intervals. These intervals were used to obtain 2-tailed *P*-values. Results of this analysis indicate that speciation and extinction rates were both significantly higher in freshwater than marine biomes (speciation: $P = 0.0016$; extinction: $P = 0.0176$; Fig. 2). Net diversification rates and transition rates

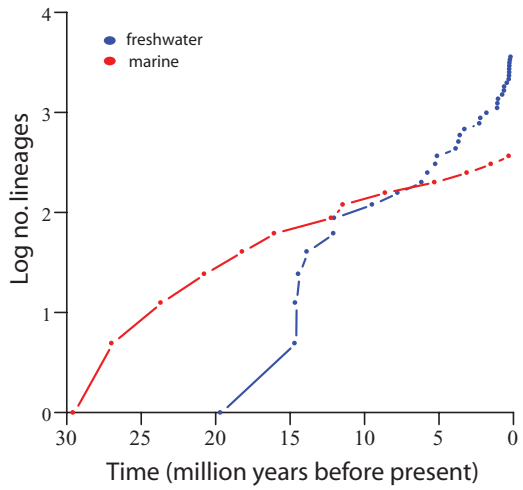

Figure 3. Lineage-through time plots (LTT) for silversides from marine (red) and freshwater (blue) habitats.

between marine and freshwater were not significantly different (net rates: $P = 0.6956$; transition rates: $P = 0.7297$).

Maximum likelihood estimates of speciation and extinction rates under GeoSSE were qualitatively the same as under BiSSE (faster speciation, extinction, and net diversification rates in freshwater vs. marine). Given the similarity in estimates, we report parameter estimates only for BiSSE (Table 3).

Our power analysis using 1700 simulated phylogenies and character datasets strongly support both the appropriateness of the best-fit BiSSE model and the utility of that model to accurately estimate parameter values. First, using AIC, the best-fit model for 76% of simulated trees was the same model that trees were simulated under, demonstrating that the various models we tested can be accurately discriminated. When using likelihood ratio tests, the model under which data were simulated rejected simpler models for 89–99.9% of simulations (depending on which models were being compared). Second, the distribution of parameter estimates under the best-fit model obtained from the simulated datasets overlapped closely with the actual parameter estimates we obtained for our phylogeny (Fig. S3). Importantly, speciation and extinction rates were significantly higher in freshwater versus marine species in our power analysis. These results indicate that not only does our silverside tree of 50 species have sufficient power to estimate a realistic model (despite lacking 24 species), but it also provides reasonable parameter estimates of both speciation and extinction.

LTT ANALYSES

Our LTT plots (Fig. 3) show that the slope of the LTT is steeper in freshwater lineages than in marine lineages, which is consistent with the faster rates of freshwater speciation estimated in our BiSSE analysis. Freshwater lineages also show a significant upturn near the present ($\gamma = 2.937$, $P = 0.0033$) that is characteristic of either high extinction rates or rapid, recent speciation rates (Nee et al. 1994; Rabosky 2006a). BiSSE estimated high background extinction in freshwater lineages suggesting that the upturn near the recent is due to extinction, and not a recent increase in speciation rates. The LTT for marine lineages shows only a slight (and nonsignificant; $\gamma = -0.577$, $P = 0.564$) downturn through time, suggesting almost constant lineage accumulation over time.

Discussion

Theory on species-area relationships (MacArthur and Wilson 1967) suggest that oceans should be more species rich than continents, and yet oceans harbor only 5–15% of all species (Vermeij and Grosberg 2010). Here we provide explicit estimates of speciation and extinction parameters using state-dependent diversification analysis to test the hypothesis that freshwater (continental) lineages have higher diversification rates than marine lineages. Our data suggest that freshwater silversides had both higher speciation rates than marine lineages, but also higher extinction rates (Table 3). The resulting net diversification rates were slightly higher in freshwater versus marine lineages, but the differences was not statistically significant. Importantly, marine silverside lineages are generally older than freshwater lineages, suggesting that freshwater lineages have had less time to generate species. We also found that freshwater silverside diversity is not due to asymmetrical transition rates between habitats, because transition rates between habitats were roughly equal and a model with transition rates constrained to be equal had almost identical likelihood and much stronger Akaike weight (Table 2). Together, these results support the idea that differences in habitats can drive macroevolutionary processes (Ribera et al. 2001; Hughes and Eastwood 2006; Alfaro et al. 2007; Moore and Donoghue 2007, 2009; Kozak and Wiens 2010) and are an important determinant of broad-scale patterns of diversity (Wiens et al. 2011). Below we discuss the evidence and possible causes for the differences in speciation and extinction rates between aquatic habitats.

SPECIATION RATES ELEVATED IN FRESHWATER

Our results confirm that speciation rates are higher in freshwater than marine silverside lineages, showing a 15-fold difference in rate (Fig. 2; Tables 2 and 3). We propose that the most probable cause of this discrepancy is differences in the abundance of physical (vicariant) barriers that limit gene flow and promote genetic divergence. Most speciation events in fishes are likely a result of allopatric speciation via vicariance (Cracraft 1985; Lynch 1989; Coyne and Orr 2004), and the frequency of vicariant events is likely higher in freshwater than marine habitats (Strathmann 1990; May 1994; but see Paulay and Meyer 2002; Dawson and Hamner 2008). Rivers in particular are strongly influenced by geological events that cause stream capture or drainage

subdivision, which are widely recognized causes of vicariant events in freshwater fishes (Rosen 1978; Mayden 1988; Waters et al. 2001; Burridge et al. 2006; Albert and Carvalho 2011). Freshwater habitats are often highly fragmented even on small spatial scales (i.e., within drainages, or between adjacent drainages; Burridge et al. 2008), resulting in high levels of microendemism and population structure (Hollingsworth and Near 2009; Keck and Near 2010), which may lead to elevated speciation rates. In addition, the high degree of habitat complexity in freshwater systems likely facilitates local adaptation, a process that has been demonstrated to play an important role in the diversification of freshwater fishes (Fuller et al. 2007; Tobler et al. 2008; Plath et al. 2010; Tobler et al. 2011). Many freshwater silverside lineages show biogeographic patterns that are consistent with allopatric speciation via river basin isolation.

In contrast, oceans are more "open" and contiguously connected ecosystems (Rapoport 1994; Carr et al. 2003). Many marine fishes, including silversides (Watson 1996), have pelagic planktonic larvae that disperse long distances, and accordingly, marine species are known to have high levels of gene flow and population connectivity (Waples 1987; Palumbi 1994; Bohonak 1999; Bierne et al. 2003; Hellberg 2009; Puebla 2009). As a result, marine fishes tend to have lower levels of population structure than freshwater fishes (Ward et al. 1994; MaKinen et al. 2006). Together, high population connectivity and gene flow in marine ecosystems dampen the effects of local adaptation and impede speciation (Bierne et al. 2003). There are notable exceptions where marine species show signatures of restricted gene flow over small spatial scales (e.g., Taylor and Hellberg 2003, 2005), but many of these taxa are reef associated and might have diversification rates on par with freshwater lineages (Bellwood and Wainwright 2002; Rocha et al. 2005; Alfaro et al. 2007; Rocha and Bowen 2008; Price et al. 2010, 2011). Marine silversides in our study are not reef associated, precluding our ability to compare diversification rates between reef and freshwater lineages. Rather most marine silversides have large, coastal distributions that likely contribute to low speciation rates.

Freshwater silversides from lakes of the Central Mexican Plateau (*Chirostoma* and *Poblana*) may represent a "species flock" that resulted (in part) from intra-lacustrine sympatric speciation (Echelle 1984). It is possible that the estimate of overall speciation rate in freshwater silversides is strongly influenced by rapid speciation in this clade. Although some studies suggest that allopatric speciation may be slower than sympatric speciation (McCune and Lovejoy 1998), others have demonstrated that allopatric speciation can occur just as rapidly, even on par with clades thought to be classic examples of sympatric speciation (Near and Benard 2004). If the latter is true, then the prevalence of a particular mode of speciation on continents or in oceans may not be a good predictor of disparity in species richness. To our knowledge, there is no study comparing the frequency of speciation modes between closely related marine and continental lineages. If sympatric speciation is more common in continental lineages, then the inclusion of a candidate clade for sympatric speciation in our study is informative and a critical component of a complete explanation for why continental species richness is high.

EXTINCTION RATES ELEVATED IN FRESHWATER

Extinction rates in freshwater silverside lineages were estimated to be almost 60 times higher than in marine lineages (Fig. 2b and Table 3). However, despite high extinction rates in freshwater lineages, net diversification (speciation rate minus extinction rate) is still 1.2 times higher in freshwater lineages, suggesting that high rates of faunal turnover in freshwater have not suppressed net diversification. Extinction rates estimated from molecular phylogenies are problematic (Rabosky 2010) and must be interpreted with caution. Nonetheless, our results for higher extinction rates in freshwater lineages are both biologically plausible and intriguing.

We propose that habitat connectivity and the ability to move in response to environmental disturbance may be key parameters that result in differences in extinction rates between marine and freshwater lineages (Jablonski 2008, and references therein). Marine habitats are stable, long lasting, and have high levels of connectivity (Lee and Bell 1999). In contrast, freshwater habitats are highly compartmentalized and spatially fragmented, with connectivity among drainages (and populations) dictated by the geomorphology of the region (Carr et al. 2003). Rivers and streams in particular have unique spatial structuring and ecosystem dynamics because they are dendritic networks, with drainages separated by uninhabitable (terrestrial and marine) areas (Grant et al. 2007). In the event of environmental disturbance (e.g., temperature change), marine fishes may be able to track suitable habitat because there are fewer barriers and physical restrictions to geographic species range shifts. For instance, during cooler periods tropical species occurring at higher latitudes might shift their ranges, moving to warmer latitudes near the equator (assuming biotic interactions allow it). Meanwhile, the fragmented nature of freshwater habitats reduces the possibility of range shifts in response to environmental disturbance such as climate change and marine incursions. Freshwater species may not have the necessary inter-drainage connections for dispersal, putting them at a higher risk of extinction (Fagan 2002; Fagan et al. 2002; Carr et al. 2003).

Both species range size and population size may also affect extinction rate. An inverse relationship between extinction rate and range size is widely supported by empirical and theoretical evidence (Jablonski 1987, 2007, 2008; Jablonski and Hunt 2006; Eastman and Storfer 2012), indeed range size is frequently cited as a likely cause of differential survival at the

species level (i.e., species selection; Rabosky and McCune 2010). Marine silverside species tend to have large ranges whereas freshwater species are narrowly distributed, often limited to a single river drainage (Barbour 1973a; Chernoff 1986b); this pattern is likely general among teleost fishes. Numerically large populations are also expected to have lower extinction rates (Jablonksi 2008). Although there are no data comparing population sizes in silversides, marine taxa likely have larger populations, which may further reduce extinction risk. Our finding of higher extinction rates in freshwater compared to marine lineages may be a widespread pattern among aquatic taxa. If so, this finding has significant implications for both macroevolutionary patterns and conservation.

GEOGRAPHIC PATTERNS OF DIVERSITY AND LINEAGE ACCUMULATION

When resources are plentiful and competitors are limited, lineages are expected to accumulate exponentially through time (e.g., constant rates). As diversification continues and niche space fills, resources become limited and competition increases, resulting in diversity-dependent diversification and a slowdown (downturn) in lineage accumulation (Weir 2006; Rabosky and Lovette 2008b; Rabosky 2009a,b). Benton (2001, 2009) Because marine habitat is ancestral in silversides, it might be expected that diversity-dependent processes have regulated diversity here, whereas the more recently derived continental diversity may not yet have been subjected to ecological constraints on clade growth. However, our results showed that neither continental nor marine silversides showed a significant pattern of diversity-dependent lineage accumulation (Fig. 3). Instead marine lineages fit a constant growth (exponential) pattern of lineage accumulation, whereas continental lineages showed a significant upturn near the present in LTT plots. The increase near the present in the freshwater LTT plot (and γ value) is consistent with our estimated high background extinction rates. This further suggests that our estimated extinction rates are plausible despite the difficulty associated with estimating extinction rates.

The nearly straight (marine) or upturned (freshwater) LTT slopes suggest that silversides have not diversified to the point of saturating niche space in either environment (although diversification seems to be more limited in eastern North America as discussed later). We find that marine lineage accumulation plots have a shallower slope than freshwater lineages, which is consistent with our hypothesis that marine lineages accumulate at a slower rate due to lower diversification rates. The few studies to investigate patterns of lineage accumulation in marine fishes have shown mixed results, but have focused on clades that are reef associated and thought to be examples of marine adaptive radiations (Ruber et al. 2003; Ruber and Zardoya 2005; Alfaro et al. 2007; Cowman and Bellwood 2011). As discussed earlier, it is likely that reef-associated clades have different diversification dynamics than nonreef-associated clades. We suspect that the processes determining marine silverside diversification are likely shared with other near-shore nonreef marine fishes, and that ecological limits are not controlling clade growth in these taxa.

The geography of marine to freshwater transitions has played an important role in shaping patterns of diversity of silversides, as well as other fishes including anchovies (Bloom and Lovejoy 2012), needlefishes (Lovejoy and Collette 2001), and sea catfishes (Betancur-R et al. 2012). Silversides independently invaded freshwaters in eastern North America/Central Mexican Plateau, southern Mexico/Central America (twice), and the Pacific coast of Colombia. However, freshwater silverside diversity is not evenly distributed among these regions (Fig. 1). Diversity is low in Colombia (one species) and eastern North America (four species, of which only *Labidesthes sicculus* has invaded far beyond lowland coastal rivers), and high in the Central Mexican Plateau (>19 species) and southern Mexico/Central America (>13 species). Overall, the fish fauna of eastern North America is very diverse (more so than southern Mexico/Central America and the Central Mexican Plateau region), and probably has been for a long time (Cavender 1986; Wilson and Williams 1992; Near et al. 2003). Both the initial invasion and subsequent diversification of lineages that have transitioned from marine to freshwater habitats may depend on the amount of competition with the incumbent freshwater community (Vermeij and Dudley 2000; Lovejoy et al. 2006; Betancur-R 2010; Yoder et al. 2010; Bloom and Lovejoy 2011, 2012; Betancur-R et al. 2012). We propose that competition with older, more diverse groups has prevented silversides from extensive diversification in North America. In contrast, the Central Mexican Plateau region has a relatively depauperate fish fauna and lacks large predatory fishes (Miller et al. 2005). Our time-calibrated tree also suggests that silversides were present in southern Mexico and northern Central America prior to the formation of the Panamanian Isthmus; thus, silversides were able to diversify before many South America fishes colonized Central America (Albert and Reis 2011). We suggest that in both the Central Mexican Plateau and southern Mexico/Central American there was ample ecological opportunity for diversification due to reduced competition with incumbents, which explains the greater freshwater silverside diversity in these regions (Betancur-R et al. 2012; Bloom and Lovejoy 2012). However, we acknowledge that testing for past competition is difficult, and future studies on past competition in silversides would benefit from explicit tests (i.e., Betancur-R et al. (2012). Our results highlight the usefulness of marine/freshwater sister lineage comparisons for understanding the effect of habitat on lineage diversity.

Conclusion

Our study demonstrates that clades of fishes that include both marine and freshwater members are excellent systems for studying macroevolutionary processes that determine patterns of species richness across oceans and continents. We have shown that transitions from marine to freshwater result in accelerated speciation and extinction rates (and to a lesser extent, net diversification rates), and that these rate differences may help explain the remarkable disparity in species richness between continents and oceans. The greater number of barriers in freshwater habitats relative to marine habitats likely results in more frequent allopatric speciation events. The higher extinction rates in freshwater habitats is likely due to variation in habitat stability and dynamics of living in an open (marine) versus restricted (freshwater) system. We suggest that elevated diversification rates of continental lineages might be a widespread phenomenon that contributing to the uneven distribution of species richness between continents and oceans.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Summary of valid species in the family Atherinopsidae. Species that were not available for DNA sequencing were incorporated into diversification analyses when indicated.

Figure S1. Bayesian phylogeny of Atherinopsidae inferred from the program MrBayes.

Figure S2. Time-calibrated phylogeny of New World Silversides (Atherinopsidae) estimated in the program BEASTv1.6.1.

Figure S3. Posterior probability parameter estimates from the power analyses. The distributions are from 1700 simulated trees and associated character data.