

UNIVERSITY OF OKLAHOMA
GRADUATE COLLEGE

CONSEQUENCES OF HABITAT TRANSITIONS ON LOCOMOTIVE TRAIT
DIVERSIFICATION AT THE MICRO- AND MACRO-EVOLUTIONARY SCALES

A DISSERTATION
SUBMITTED TO THE GRADUATE FACULTY
in partial fulfillment of the requirements for the
Degree of
DOCTOR OF PHILOSOPHY

By
JAMES C. CURETON II
Norman, Oklahoma
2015

CONSEQUENCES OF HABITAT TRANSITIONS ON LOCOMOTIVE TRAIT
DIVERSIFICATION AT THE MICRO- AND MACRO-EVOLUTIONARY SCALES

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

Dr. Richard E. Broughton, Chair

Dr. Ben F. Holt III

Dr. John P. Masly

Dr. William J. Matthews

Dr. Gary A. Wellborn

DEDICATION

I dedicate this thesis to my grandparents, whom I wish could have been here to see me receive my doctoral degree.

ACKNOWLEDGMENTS

Completion of this dissertation would not have been possible without the help of several people. This would not have been possible without my wife's care and understanding for the time I dedicated to complete this research and this dissertation. I received logistical and technical assistance from a small army including Aaron Geheber, Zach Zbinden, Bryan Frenette, Chris Cureton, and Brent Tweedy. Edie-Marsh Matthews and Sara Cartwright provided unabated access to the fish collections at the Sam Noble Museum of Natural History and Justin Mann provided access to the collections at the Tulane University Biodiversity Research Institute. My advisor graciously helped mold my research and provided significant constructive criticism that guided the completion of this research. My other committee members, Bill Matthews, JP Masly, Gary Wellborn, and Ben Holt, provided constructive criticism that guided my research and significantly improved the quality of the manuscripts herein.

These studies could not have been completed without funding from the Society for the Study of Evolution (Rosemary Grant Graduate Student Research Award), the University of Oklahoma Graduate Student Senate, and the University of Oklahoma Department of Biology (Loren Hill Award). All of the research conducted herein was approved by the University of Oklahoma Institutional Animal Care and Use Committee (IACUC-R12-015).

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	vii
LIST OF FIGURES.....	ix
ABSTRACT.....	xiv
CHAPTER 1.....	1
ABSTRACT.....	2
INTRODUCTION.....	3
METHODS.....	4
RESULTS.....	5
DISCUSSION.....	6
REFERENCES.....	8
CHAPTER 2.....	14
ABSTRACT.....	15
INTRODUCTION.....	16
METHODS.....	17
RESULTS.....	20
DISCUSSION.....	21
REFERENCES.....	25
CHAPTER 3.....	31
ABSTRACT.....	32
INTRODUCTION.....	33
METHODS.....	35
RESULTS.....	39

DISCUSSION.....	42
REFERENCES.....	46
APPENDIX.....	57

LIST OF TABLES

TABLE	PAGE
1	Collection localities, stream-reservoir pair, collection numbers (OKMNH), year of collection (Year Col), number of years following impoundment (Year Imp), rate of change in Haldanes (H), and sample size (n). A negative number in the “Year Imp” column indicates the collection occurred prior to reservoir construction.....11
S1	Species, locality, Sam Noble Museum of Natural History collection number (OKMNH), year of collection (Year Col), number of years following stream impoundment (Year Imp), and sample size (n) for each collection used in this study.....58
S2	F-value, degrees of freedom (df), and P-value from statistical analyses (univariate or multivariate analyses of variance) comparing size, body shape, or caudal fin aspect ratio stream and reservoir populations.....61
S3	The mean rate of change in Haldanes (H) and 1 standard error (SE) for each trait in the eight cyprinid species included in this study. The number of years (N) over which the rate of evolution was estimated for each trait are indicated. Rates that were included in statistical analyses are indicated in bold.....62
S4	The percent of variation explained by each informative PC and the cumulative variance explained by the informative PC for each species.....63

S5 Species used in the morphological analyses, the museum collection number for the borrowed specimens (Oklahoma Museum of Natural History [OKMNH] or Tulane University Biodiversity Research Institute [TUBRI]), number of specimens used in the analysis, habitat classification, “phylogenetic pair” for convergent evolution analyses, and NCBI GenBank accession number for the COI, CYTB, S7, and RAG1 genes for each species. Specimens not included in convergent evolution analyses are indicated by “-“ as are genes not available for a particular species.....64

LIST OF FIGURES

FIGURE		PAGE
1	<p>Illustration of body shape differences among stream (a) and reservoir (b) fish by thin plate spline visualization of variation along PC1. Stream fish tended to have more negative PC1 scores than reservoir fish with notable change in the terminality of the head, the location of the dorsal and pelvic fins, and body depth.....</p>	12
2	<p>(a) Comparison of morphological differences (PC1) for the stream (filled circles) and reservoir (open circles) fish for seven stream-reservoir pairs. The circle with an \times indicates the reservoir samples from Lake Texoma. Error bars are 1 SE. (b) Average PC1 scores of Red River and Lake Texoma populations plotted by the time under reservoir conditions. The regression line (solid) and upper and lower 95% confidence intervals from bootstrap resampling (dotted lines) are shown. (c) The standardized selection differential (filled circles) and index of stabilizing selection (empty circles) for each Lake Texoma population plotted by the time under reservoir conditions. The solid line at $Y=0$ identifies the point when directional selection transitions to stabilizing selection ($j<0$) based on the index of stabilizing selection.....</p>	13
3	<p>Summary of the direction of change in body size, body shape, and caudal fin shape for all eight species. The arrow indicates the direction of change for body size or caudal fin aspect ratio and the direction of change in body shape is indicated by a wireframe grid with vectors</p>	

representing the direction and magnitude of change in each landmark.

The direction of change for all traits is from the stream population to the reservoir population.....29

4 Average rate of evolution in Haldanes (mean \pm 1 standard error) for each trait (a) and species (b). Only traits that showed a response to impoundment are included.....30

5 Reconstruction of benthic (black) and pelagic (dark grey) habitats on the Bayesian phylogenetic hypothesis (with the four major clades indicated). The hypothesis was trimmed to include only the species for which we had morphometric data. The “phylogenetic pairs” used in the convergent evolution analyses are indicated by a numbered circle. The species belonging to each “phylogenetic pair” are provided in Table S1.....53

6 Thin plate splines illustrating body shapes that correspond to variation in PC2 (a) and PC4 (b) for benthic and pelagic species. Benthic species tended to have larger heads and deeper bodies than their pelagic counterparts which had smaller heads and streamlined bodies.54

7 Average PC2 and PC4 scores (mean \pm 1 standard error) for all benthic (filled circles) and pelagic (empty circles) species as well as the convergent benthic species (filled square) and their sister pelagic species (empty square) that composed the “phylogenetic pairs.”55

8 Scatterplot of mean PC2 and PC4 scores for each benthic (filled circles) and pelagic (empty circles) species included in the analysis of benthic

	and pelagic morphospaces. 95% confidence ellipses are shown for benthic (solid line) and pelagic (dashed line) species.	56
S1	Eigenvalues (inertia) of each of the first principal components is indicated by the grey bars. The individual points on the red line indicate the eigenvalues of a randomly generated data set of equivalent size. Principal components are of interest only when the eigenvalue from the dataset is larger than the eigenvalue of the randomly generated data set. This occurs only for PC1 which is why we used only PC1 in the manuscript.....	71
S2	Body shape differences of the natural populations examined here compared with experimental results. Experimental <i>P. vigilax</i> juveniles (N=58) were collected from the Washita River (which empties into Lake Texoma) and raised in artificial mesocosms with standing (n=29) or flowing (n=29) water for 30 days. The flow rate averaged approximately 0.5 m/s in the mesocosms with flowing water, a rate which is comparable to their native habitat and other streams in Oklahoma. Shown are mean scores for the PC1 vector for the stream (filled) and reservoir (open) locations of the seven stream-reservoir pairs and plasticity data for fish raised in standing (open) and flowing (filled) water conditions.....	72
S3	GPS-referenced Sam Noble Museum of Natural History collections of <i>C. lutrensis</i> (a), <i>C. venusta</i> (b), <i>C. carpio</i> (c), <i>H. placitus</i> (d), <i>M. storeriana</i> (e), <i>N. potteri</i> (f), <i>N. atherinoides</i> (g), and <i>P. vigilax</i> (h) in Lake Texoma. The Red River enters Lake Texoma on the west (left in the pictures) and	

	Washita River enters Lake Texoma on the north (top of the pictures).	73
S4	Intensity of selection (mean \pm 1 standard error) estimated for each trait that demonstrated a response to stream impoundment. The intensity of selection was estimated from the stream population to the first population that achieved the reservoir phenotype.....	74
S5	Intensity of selection (mean \pm 1 standard error) estimated for each species. <i>Hybognathus</i> was not included in a formal statistical analysis because only one trait exhibited a response to stream impoundment. Only traits that showed a response to impoundment were included for each species.....	75
S6	Phylogenetic hypothesis recovered from maximum likelihood analysis of the two nuclear and two mitochondrial genes for 269 species of North American cyprinids. Bootstrap support at each node is presented as a percentage.....	76
S7	Phylogenetic hypothesis recovered from Bayesian analysis of the two nuclear and two mitochondrial genes for 269 species of North American cyprinids. Support at each node is presented as a probability.....	77
S8	Reconstruction of habitat type on the maximum likelihood topology recovered approximately 25.4 transitions between habitat types (Figure S3). Transitions from pelagic to benthic habitats (18.0 transitions) were more common than transitions from benthic to pelagic habitats (7.4	

transitions). Benthic habitats are indicated in black and pelagic habitats
are indicated in grey.....78

ABSTRACT

Contemporarily evolving systems provide a unique opportunity to characterize the direction, pattern, and rates of phenotypic change among multiple species. The damming of streams to create reservoirs results in a change from lotic to lentic environments and induces the evolution of phenotypes that optimize unsteady swimming performance in fishes. In my first chapter, I examined the spatial and temporal patterns of evolution of body shape in a widespread North American stream fish (*Pimephales vigilax*) in response to stream impoundment. I observed significant changes in body depth, head shape, and fin placement following dam construction in each of seven different Oklahoma rivers. The magnitude of change was greatest in the first 15 generations post-impoundment, followed by continued but more gradual change thereafter. In my second chapter, I compare the direction and rate of evolution of body size, body shape, and caudal fin shape following stream impoundment in eight cyprinids. My data show that these traits do not diverge in the same direction in all species. Rates of evolution during the period of directional selection were consistent among traits and species, perhaps due to similar amounts of underlying quantitative genetic variation as a result of their recent common ancestry. All of the above results indicate that changes in environmental conditions can result in rapid evolution of functionally important traits.

In my third chapter, I tested the hypothesis that transitions between benthic and pelagic habitats resulted in convergent evolution of body shape in North American cyprinids. I built a phylogenetic hypothesis of 201 species using four genes, reconstructed the evolution of habitat type on the recovered topology, and evaluated

evolution of body shape in benthic and pelagic species. I detected approximately 25 transitions between benthic and pelagic species with pelagic-to-benthic transitions being most common. Body shape was significantly different between benthic and pelagic species with benthic species developing deeper bodies with larger heads than pelagic species. However, benthic and pelagic species did not inhabit mutually exclusive regions of morphospace suggesting that convergent evolution of body shape is incomplete in this group of fishes. My results show that habitat transitions can drive convergent evolution of similar phenotypes among distinct evolutionary lineages. However, the magnitude of convergence among different lineages is likely constrained by their unique evolutionary history.

**CHAPTER 1: RAPID MORPHOLOGICAL DIVERGENCE OF A STREAM
FISH IN RESPONSE TO CHANGES IN WATER FLOW¹**

¹Cureton II, J.C. and R.E. Broughton. 2014. Rapid morphological divergence of a stream fish in response to changes in water flow. *Biology Letters* **10**, 20140352. (doi: <http://dx.doi.org/10.1098/rsbl.2014.0352>)

ABSTRACT

Recent evidence indicates that evolution can occur on a contemporary time scale. However, the precise timing and patterns of phenotypic change are not well known. Reservoir construction severely alters selective regimes in aquatic habitats due to abrupt cessation of water flow. We examined the spatial and temporal patterns of evolution of a widespread North American stream fish (*Pimephales vigilax*) in response to stream impoundment. Gross morphological changes occurred in *P. vigilax* populations following dam construction in each of seven different rivers. Significant changes in body depth, head shape and fin placement were observed relative to fish populations that occupied the rivers prior to dam construction. These changes occurred over a very small number of generations and independent populations exhibited common responses to similar selective pressures. The magnitude of change was observed to be greatest in the first 15 generations post-impoundment, followed by continued but more gradual change thereafter. This pattern suggests early directional selection facilitated by phenotypic plasticity in the first 10 – 20 years, followed by potential stabilizing selection as populations reached a new adaptive peak (or variation became exhausted). This study provides evidence for rapid, apparently adaptive, phenotypic divergence of natural populations due to major environmental perturbations in a changing world.

INTRODUCTION

A fundamental concept in evolutionary biology is that organismal phenotypes change in response to changes in their environment. The perception that evolutionary change is relatively slow, taking hundreds to thousands of generations is yielding to evidence of phenotypic changes on contemporary time scales [1-3]. Although examples of rapid evolution are increasingly common, only rarely is the precise timing of environmental change and organismal response well resolved outside of the laboratory. Consequently, details of phenotypic divergence at the upper end of the evolutionary rate scale remain unclear in natural populations.

In nature, the strength and direction of selection can vary with annual cycles [4] and phenotypic plasticity may contribute to abrupt patterns of phenotypic change [5]. Habitat alterations, such as dam-formed reservoirs, provide excellent opportunities to study temporal aspects of divergence because they cause temporally defined and permanent shifts from lotic (riverine) to lentic (lake) habitats [6]. In some fishes, populations sampled from lotic and lentic habitats exhibit significant morphological differences: development of a deeper body in lake-dwellers than stream-dwelling conspecifics [3, 7]. The deeper body phenotype is presumed to be adaptive because it can enhance swimming burst speed and maneuverability relative to a more streamlined phenotype which may better maintain position in steady current [8-9]. Such adaptive changes could presumably evolve quickly if there is strong selection on one or more of the major or minor quantitative trait loci (QTL) that underlie body shape [10].

We investigated body shape change before and after river impoundment in multiple populations of a widespread stream fish (*Pimephales vigilax*, Cyprinidae) and

assessed the timing of phenotypic divergence in a lentic reservoir environment.

Morphometric analyses revealed similar rapid changes in body shape after reservoir construction across all populations. These results highlight the potential for significant phenotypic change over only a few generations in nature.

METHODS AND MATERIALS

We sampled *P. vigilax* in the Sam Noble Oklahoma Museum of Natural History from collections made prior to and after impoundment of seven Oklahoma streams. Samples consisted of collections of fish from reservoirs paired with collections from free-flowing adjacent reaches of the same stream (Table 1). We photographed the left lateral side of each specimen and assigned 14 homologous landmarks to each photograph with *tpsDig* [11]. We removed variation due to specimen rotation, transformation, and scaling using a General Procrustes Analysis, condensed landmarks into fewer meaningful variables using a principal components (PC) analysis, and determined the number of relevant PC using the broken stick method [12].

We compared the first PC, which was identified as the only PC of interest (Supplementary Material; Figure S1), using a mixed-effects model. Since we were specifically interested in comparing body shape of fish from stream and reservoir habitats, we treated habitat (stream or reservoir) as a fixed factor and location (stream-reservoir pair) and the habitat \times location interaction as random factors after accounting for size allometry (centroid size) [13]. Because of the relatively large number of Lake Texoma samples relative to the other populations, we used only one randomly selected sample to represent Lake Texoma (OKMNH #40615) in this model. Significance of

each term was determined using a chi-square test and the amount of change of each reservoir population was quantified relative to the stream population in Haldanes [2].

Because we had multiple reservoir samples for Lake Texoma, we compared PC1 of these samples across years (treated as a random effect) after accounting for allometric effects using a mixed effects model. The overall rate of change in Lake Texoma was estimated in Haldanes using the regression approach [2]. To assess the timing of phenotypic change in the Lake Texoma population, body shape was related to the amount of time the population experienced lentic conditions (time since dam construction). We accounted for unequal sample sizes among collections by bootstrapping the body shape data for each sample 100 times and performed regressions on each bootstrap replicate using the average F-statistic, p-value, and correlation coefficient. We calculated the standardized selection differential (i) to determine if the resulting pattern was due to a reduction in the strength of directional selection over time [15]. Finally, we estimated the index of stabilizing selection (j) to determine if a reduction in directional selection was associated with potential stabilizing selection ($j < 0$) [15, but see 16]. All statistical analyses were performed in R v.3.0.2 [17].

RESULTS

PC1 explained 20.2% of the variation in landmarks with notable shifts in the terminality of the head, the location of the dorsal and pelvic fins, and body depth (Figure 1). Size did not account for a significant portion of the variation in body shape ($X^2=0.155$, $P=0.694$). There was a significant habitat \times location interaction ($X^2=7.673$, $P=0.006$), but reservoir pair was not a significant random effect ($X^2=0.265$, $P=0.607$). PC1 varied in parallel across all stream-reservoir pairs ($X^2=16.450$, $P<0.001$), with

reservoir populations having larger scores than stream populations (Figure 2a). The rate of change varied from -0.068 H to 0.160 H across reservoirs, but tended to decline over time in the Lake Texoma population (Table 1).

After accounting for allometric effects in the Lake Texoma populations ($X^2=49.124$, $P<0.001$), body shape still varied significantly across collection years ($X^2=10.900$, $P=0.001$). Specifically, PC1 increased logarithmically with the number of years following river impoundment, as body shape was deeper in fish experiencing lentic conditions ($F_{1,13}=6.485$, $P=0.043$, $R^2=0.319$; Figure 2b). The average rate of change in the Lake Texoma population was 0.0129 ± 0.0125 (2 standard errors), an estimate lower than the rate of change at any particular time in Lake Texoma. Standardized selection differentials indicated that directional selection tended to increase through the first 20 generations before leveling off (log-transformed: $F_{1,13}=7.336$, $P=0.018$, $R^2=0.312$; Figure 2c). In contrast, the index of stabilizing selection exhibited a marginally significant quadratic relationship with time suggesting that potential stabilizing selection was the strongest at 12 – 30 years, but weakened thereafter ($F_{1,13}=3.542$, $P=0.062$, $R^2=0.266$; Figure 2c).

DISCUSSION

We investigated the pattern and tempo of body shape divergence following river impoundment in *P. vigilax*. We show that morphological changes, including head size and shape, dorso-ventral body depth, fin positions, and caudal peduncle thickness, occurred in all populations after abrupt changes in flow regime. The response of each population was similar in direction, indicating common solutions to a similar selective pressure [3]. The highest rate of change observed in this study (0.160 H, Lake Wister) is

comparable to rates of change observed in quantitative traits of other organisms, e.g. bill length in American house sparrows [2]. Time-series data for Lake Texoma, indicate the highest rate of change occurred in the sample at year 11, after which, rates of change declined substantially. The decline could be indicative of exhaustion of genetic variation in QTLs that underlie body shape. Alternatively, inference of standardized selection differentials suggest this pattern may be due to directional selection in the first 10 – 20 years followed by potential stabilizing selection which maintained the population on a new adaptive peak in the lotic environment [18].

Phenotypic plasticity may have contributed to the initial shape change. In a related study, we demonstrated that stream-derived juvenile *P. vigilax*, when experimentally raised in standing water, develop significantly deeper body morphs than adults from their source population (Supplementary Material; Figure S2). However, the magnitude of that change was only a small fraction of the total change observed in the present study. Phenotypic plasticity is the ability of one genotype to produce more than one phenotype under different environmental conditions [5] and, by itself, would not account for incremental change observed over several generations. However, phenotypic plasticity accompanied by directional selection may best explain this pattern of divergence. We note that in this case positive selection could be acting on DNA sequence variation or it could act to increase phenotypic plasticity in the direction of selection [19] (possibly including epigenetic variation).

The timing and pattern of morphological divergence in *P. vigilax* appears to be best explained by an initial shift due to phenotypic plasticity followed by rapid but none-the-less incremental and adaptive change in response to the shift to a standing-

water environment. Differences in the rate and magnitude of phenotypic responses, as well as variation among stream-reservoir pairs, may have been influenced by the extent of genetic variation present in each population as well as the strength of selection based on the local flow rate, predators, and food types present at each locality. The retrospective analysis is consistent with plasticity initially maintaining viable populations in the standing-water environment until adaptive evolution can proceed. Characterization of QTL or epigenetic loci that underlie body shape variation in *P. vigilax*, and fishes in general, will have important implications for our understanding of adaptive evolution and illuminate the potential responses of organisms to a rapidly changing world.

REFERENCES

1. Carroll, S. P., Hendry, A. P., Reznick D. N., & Fox, C. W. 2007. Evolution on ecological time-scales. *Func. Ecol.*, **21**, 387-393. (DOI 10.1111/j.1558-5646.2007.00259.x)
2. Hendry, A. P., & Kinnison, M. T. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evol.*, **53**, 1637-1653.(DOI 10.2307/2640428)
3. Haas, T. C., Blum, M. J., & Heins, D. C. 2010 Morphological responses of a stream fish to water impoundment. *Biol. Lett.* (DOI 10.1098/rsbl.2010.0401)
4. Siepielski, A. M., DiBattista J. D., & Carlson, S. M. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecol. Lett.* **12**, 1261-1276. (DOI 10.1111/j.1461-0248.2009.01381.x)

5. Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. 2008. Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.*, **17**, 20-29. (DOI 10.1111/j.1365-294X.2007.03428.x)
6. Graf, W. L. 1999 Dam nation: a geographic census of American dams and their large-scale hydrologic impacts. *Water Resour. Res.*, **35**, 1305-1311. (DOI 10.1029/1999WR900016)
7. Franssen, N. R. 2011 Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evol. App.*, **4**, 791-804. (DOI 10.1111/j.1752-4571.2011.00200.x)
8. Webb, P. W. 1982 Locomotor patterns in the evolution of Actinopterygian fishes. *Am. Zool.*, **22**, 329-342. (DOI: 10.1093/icb/22.2.329)
9. Langerhans, R. B. 2008 Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comp. Bio.*, **48**, 750-768. (DOI: 10.1093/icb/icn092)
10. Albert, Y. A., Sawaya, S., Vines, T. H., Knecht, A. K., Miller, C. T., Summers, B. R., Balabhadra, S., Kingsley, D. M., & Schluter, D. 2008. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evol.*, **62**, 76-85. (DOI: 10.1111/j.1558-5646.2007.00259.x)
11. Rohlf, F. J. 2010. tpsDig2. Stony Brook University.
12. Jackson, D. A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecol.*, **74**, 2204-2214.
13. Zelditch, M. L., Swiderski, D. L., Sheets, D. H., & Fink, W. L. 2004. *Geometric morphometrics for biologists*, 2nd edn. San Diego, CA: Academic Press.

14. Scott, W. B., & Crossman, E. J. 1979 Freshwater Fishes of Canada. Gordon Soules Book Pub, Vancouver.
15. Perez, K. O., & Munch, S. B. 2010. Extreme selection on size in the early lives of fish. *Evol.*, **64**, 2450-2457. (DOI: 10.1111/j.1558-5646.2010.00994.x)
16. Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evol.*, **42**, 849-861.
17. R Core Team. 2013 R: A language and environment for statistical computing. Vienna, Austria.
18. Estes, S., & Arnold, S. J. 2007. Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *Amer. Nat.*, **169**, 227-244.
19. Garland Jr., T. & Kelly, S. A. 2006. Phenotypic plasticity and experimental evolution. *J. Exper. Biol.*, **209**, 2344-2361. (DOI 10.1242/jeb.02244)

TABLE 1

Locality	Pair	OKMNH	Year Col	Year Imp	H	n
Lake Eufaula	1	52419	1992	28	0.043	5
Canadian River	1	36101	1962	-2	NA	14
Hulah Reservoir	2	28868	1956	5	0.081	17
Caney River	2	64543	1995	44	NA	8
Lake Thunderbird	3	82210	2007	43	0.014	20
Little River	3	31898	1962	-2	NA	11
Lake Grand	4	26137	1948	8	0.111	6
Neosho River	4	67419	2001	61	NA	30
Lake Wister	5	27119	1955	6	0.160	15
Poteau River	5	75074	2004	49	NA	8
Lake Texoma	6	39442	1950	6	-0.068	3
Lake Texoma	6	28108	1953	9	0.058	11
Lake Texoma	6	27631	1954	10	0.029	15
Lake Texoma	6	27782	1955	11	0.130	9
Lake Texoma	6	30155	1956	12	0.055	9
Lake Texoma	6	30031	1958	14	0.077	19
Lake Texoma	6	31145	1959	15	0.063	25
Lake Texoma	6	39699	1962	18	0.027	19
Lake Texoma	6	39513	1963	19	0.038	17
Lake Texoma	6	40615	1971	27	0.039	20
Lake Texoma	6	44149	1989	45	0.024	18
Lake Texoma	6	48068	1993	49	0.027	11
Lake Texoma	6	63013	1995	51	0.020	23
Lake Texoma	6	60315	1999	55	0.017	20
Lake Texoma	6	62031	2000	56	0.019	23
Red River	6	80558	2010	66	NA	30
Lake Oologah	7	54061	1993	30	0.061	10
Verdigris River	7	28614	1956	-7	NA	20

FIGURE 1

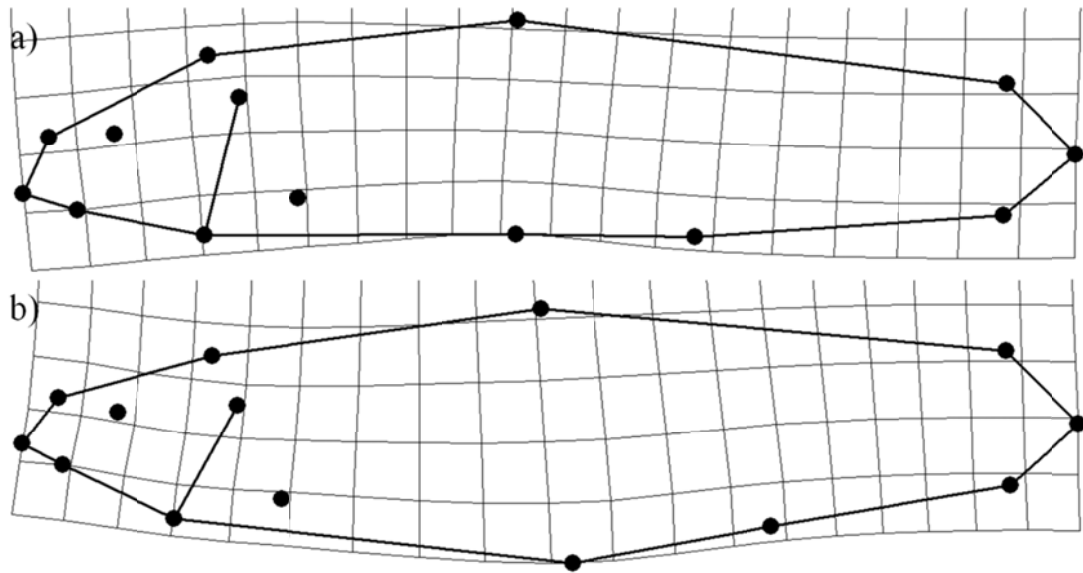
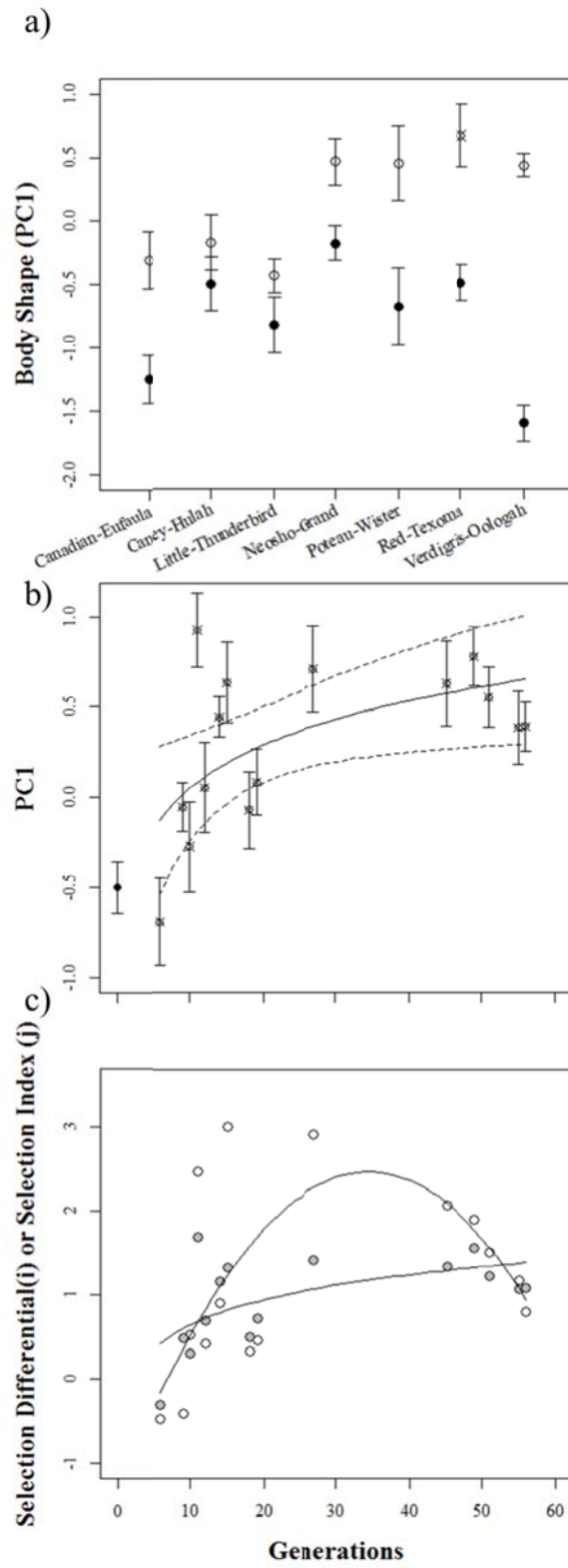


FIGURE 2



**CHAPTER 2: COMPARABLE RATES OF TRAIT AND SPECIES EVOLUTION
FOLLOWING STREAM IMPOUNDMENT²**

²Formatted for submission to *Proceedings of the Royal Society: B*

ABSTRACT

The rate at which traits evolve is a question of central importance in evolutionary biology. This question has been difficult to answer, however, because evolutionary rates should be compared from traits measured from the same population over the same time period. We took advantage of a unique dataset and compared the direction and rate of evolution of body size, body shape, and caudal fin shape in eight species of cyprinids following stream impoundment. No trait diverges in the same direction among all species although reservoir populations had deeper bodies in species collected in the reservoir lacustrine zone. Traits that responded to stream impoundment exhibited a pattern indicative of directional selection immediately followed by stasis, potentially due to stabilizing selection. Rates of evolution during the period of directional selection were consistent among traits and species. The most compelling explanation may be that a similar amount of quantitative genetic variation underlies each of these traits as a result of the recent common ancestry of these eight species. These results highlight the potential for similar rates of rapid evolution among traits and species following a single environmental change and provide the impetus to understand the underlying cause of similar rates of evolution.

INTRODUCTION

The rate at which phenotypes evolve has long interested evolutionary biologists because they are important to understanding a variety of issues in evolution including compatibility between microevolution and macroevolution (Reznick et al. 1997), the contribution of selection and drift to phenotypic evolution (Lande 1976), and the potential for species to respond to environmental changes quick enough to prevent extinction (Stockwell et al. 2003). However, precise estimates of evolutionary rates in nature are not readily available because of an inability to track rapid changes in the strength and direction of natural selection (Siepielski et al. 2009). Further complicating is that rates compared among traits or species measured over different time intervals could be misleading because the rate is not independent of the time interval over which it was measured (Gingerich 1983; Hendry and Kinnison 1999). Likewise, evolutionary rates are not independent of the populations from which they are collected due to intrinsic (e.g., phenotypic plasticity) and extrinsic (e.g., selection intensity) factors that vary across populations. An ideal approach to minimize these confounding factors would be to compare rates estimated for traits and species from the same environment and over the same time span (Quinn and Adams 1996).

Reservoirs represent a recent shift from a lotic to lentic habitat that influences a variety of plant and animal species. In fishes, this stream-to-reservoir transition selects for phenotypes that enhance burst swimming performance rather than endurance swimming performance (Langerhans 2008). Specifically, high flow environments select for a streamlined body shape which delays separation of the boundary layer from the body thereby reducing drag and the energetic expenditure necessary to maintain their

position in the water column (Webb and Buffrénil 1990). Low flow environments select for a deeper body shape which reduces recoil during swimming and enhances burst swimming performance (Webb 1982). Consequently, fishes in reservoirs frequently evolve larger heads, and posteriorly deeper bodies and caudal peduncles than their stream conspecifics (Haas et al. 2010; Franssen 2011; Franssen et al. 2013; Cureton and Broughton 2014). Other traits, such as body size and caudal fin shape, are important to locomotion and may exhibit a similar evolutionary response following stream impoundment (Sambilay 1990).

Reservoirs provide a unique opportunity to quantify the rate of change among several traits and species in response to the same selective pressure (i.e., stream impoundment). We compared rates of evolution that were estimated from the same environment and the same time period to test the hypothesis that rates of evolution vary among species and traits. We focused on the Lake Texoma, a reservoir formed by construction of Denison Dam at the confluence of the Red and Washita Rivers in 1944. By quantifying rates of evolution in response to impoundment of the Red River, we can account for temporal and spatial factors that typically confound comparisons of evolutionary rates. Using fishes collected from Lake Texoma, we characterized divergence of three locomotive traits (body size, body shape, and caudal fin shape) over 60 years in eight species of cyprinid fishes. These data show that all three traits diverged in different directions among species, but rates of evolution were similar among traits and species highlighting the need to understand why different traits and species evolve at similar rates following a change in selective pressures.

METHODS

We identified museum collections of cyprinid species (Cyprinidae) at the Sam Noble Museum of Natural History that were collected from: *i*) Lake Texoma on at least 8 occasions from 1944 to present day, and *ii*) a free-flowing reach of the Red River (Table S1). Based on the available collections, we included the red shiner (*Cyprinella lutrensis*), the blacktail shiner (*Cyprinella venusta*), common carp (*Cyprinus caprio*), plains minnow (*Hybognathus placitus*), silver chub (*Macrhybopsis storeriana*), emerald shiner (*Notropis atherinoides*), chub shiner (*Notropis potteri*), and bullhead minnow (*Pimephales vigilax*; Cureton and Broughton 2014) in this study (Table S1). Three of the eight species (*H. placitus*, *M. storeriana*, and *N. potteri*) naturally prefer medium to large rivers and are largely collected at the inflows of the Red and Washita Rivers (Figure S1; Riggs and Bonn 1959). Any sustainable populations of these species may live in Lake Texoma's riverine and transitional zones (Thornton et al. 1990). The other five species are common to natural pools and reservoirs, collected ubiquitously throughout the reservoir (Figure S3), and as such, may have stable populations in the reservoir's riverine, transitional, and lacustrine zones (Thornton et al. 1990).

We photographed the left lateral side of 1-30 specimens from each museum collection using a Sony DSLR-A350 camera (Table S1). We used geometric morphometric techniques to assess body size and shape (Zelditch et al. 2004). We assigned 14 homologous landmarks to each photograph using TPSdig2, aligned the landmarks separately for each species using a General Procrustes Analysis in the "geomorph" package, and then subjected to a principal component (PC) analysis to condense the landmarks into fewer, meaningful variables (Figure 1; Rohlf 2010; Adams and Otárola-Castillo 2013). Centroid size was used as a surrogate for body size and the

number of informative body shape PC was determined using the broken-stick method (Jackson 1993). We assessed caudal fin shape using aspect ratio which is a measure of the height (h) of the un-stretched caudal fin relative to the area (a). To estimate aspect ratio, we imported each photograph into Adobe Photoshop[®] (San Jose, California) and measured h and a of the lower caudal fin lobe in pixels (the upper lobe was degraded in the majority of specimens). Because we measured h and a for only the lower lobe, we calculated aspect ratio of the using the modified formula $CFAR=2h^2/(2a)$ (Sambilay 1990).

We assessed divergence between stream and reservoir populations by comparing the Red River population to the most recent Lake Texoma collection (Table S1). We compared body size of each species between *habitats* using a type-III analysis of variance (ANOVA), body shape of each species using a type-III multivariate analysis of covariance (MANCOVA), and caudal fin aspect ratio using an analysis of covariance (ANCOVA). We included *habitat* as a fixed factor in all analyses and centroid size as a covariate in analyses of body shape and caudal fin aspect ratio.

We considered traits to show a response to impoundment if *i*) the stream phenotype was significantly different from the most recently sampled reservoir collection and *ii*) the trait exhibited a directional phenotypic response after impoundment followed by a period of stasis (Cureton and Broughton 2014). For traits that exhibited a response to impoundment, we estimated the rate of evolution in Haldanes (H) during the period of directional selection using the linear regression approach (Hendry and Kinnison 1999). Because estimating rates of evolution in H requires generation times, we used the following estimates of minimum age at

reproduction as generation times: *C. lutrensis*, *C. venusta*, *H. placitus*, *M. storeriana* (based on *Macrhybopsis aestivalis*), and *P. vigilax* – 1 year; *N. atherinoides* – 2 years; *C. carpio* – 3 years (Scott and Crossman 1979; Becker 1983; Mayden 1991; Robinson and Buchanan 1992; Jenkins and Burkhead 1994). No published data are available for *N. potteri*; therefore, we used the most conservative estimate of 1 year. Because we were interested in only the rate and not the direction of evolution for these comparisons, we used the absolute value of the rate. We compared rates among traits and species using a “type-III” ANOVA. All statistical analyses were performed in R v.3.1.1 Statistical Software (R Core Team; Vienna, Austria).

RESULTS

Reservoir fish were significantly larger than stream fish in *C. lutrensis*, *H. placitus*, *M. storeriana*, and *N. atherinoides* whereas reservoir *N. potteri* and *P. vigilax* were significantly smaller than their stream conspecifics (Figure 3; Table S2). Body size showed a response to stream impoundment in five species with the transition from stream-to-reservoir body size occurring on average in 14.1 ± 8.8 generations, although *H. placitus* increased this time substantially (49.0 generations in *H. placitus* vs. 5.4 generations in four other species; Table S3). The average rate of body size evolution in these five species was 0.229 ± 0.069 H (Table S3).

Forty-one total body shape PC were informative and included in subsequent analyses (Table S4). Body shape was significantly different between stream and reservoir populations for all species except *H. placitus* and *M. storeriana* (Table S2). Reservoir *C. lutrensis*, *C. venusta*, and *P. vigilax* had shorter heads, deeper bodies and caudal peduncles, and posterior shifted dorsal fins relative to their stream conspecifics

(Figure 3). Reservoir *C. carpio* had larger heads, shallower bodies and caudal peduncles, posterior shifted dorsal fins, and dorsally shifted anal fins whereas *N. atherinoides* and *N. potteri* developed smaller heads, shallower bodies, and an anterior or posterior shifted dorsal fin (Figure 3). The average time it took to reach the reservoir phenotype for the 19 body shape PC that showed a response to stream impoundment was 16.6 ± 4.0 generations, evolving at an average rate of 0.237 ± 0.067 H (Table S3).

Caudal fin aspect ratio was significantly different between stream and reservoir fish in *C. lutrensis*, *C. venusta*, *N. potteri*, and *P. vigilax*. Reservoir *C. lutrensis*, *C. venusta*, and *P. vigilax* had higher aspect ratio caudal fins than their stream conspecifics, whereas reservoir *N. potteri* had lower aspect ratio caudal fins than their stream counterparts (Figure 3). Only aspect ratio of *P. vigilax* showed a clear evolutionary response to impoundment and evolved at a rate of 0.219 H over 14 generations (Table S3).

Because caudal fin aspect ratio in only *P. vigilax* exhibited an evolutionary response to impoundment, it was removed from subsequent analyses. Likewise, *H. placitus* was removed from the analysis because only body size showed an evolutionary response. When we included all other traits that showed an evolutionary response to stream impoundment, rates were not significantly different among traits ($F_{1,16}=0.102$, $P=0.753$, $\eta^2=0.006$) or species ($F_{6,16}=0.982$, $P=0.469$, $\eta^2=0.269$) (Figure 4). These results did not change if all traits (i.e., including those that did not show a “clear” evolutionary response to stream impoundment) were included in the analysis (traits: $F_{2,47}=0.588$, $P=0.560$, $\eta^2=0.024$; species: $F_{7,47}=1.037$, $P=0.419$, $\eta^2=0.134$).

DISCUSSION

Comparisons of evolutionary rates historically have been difficult because of temporal and spatial factors that confound such comparisons, such as the non-independence of rates of evolution and the time span over which they are measured. We compared rates of evolution of three locomotive traits in eight cyprinids following impoundment of the Red River, a unique approach that minimizes confounding temporal and spatial factors. We observed divergence of body size, body shape, or caudal fin shape following stream impoundment in all species, but these traits did not always diverge in the predicted direction. Body size increased in one species, body depth increased in five species, and caudal fin aspect ratio decreased in one species despite low flow environments favoring phenotypes that result in enhanced unsteady swimming performance, most notably increased body size and body depth and decreased caudal fin aspect ratio (Webb 1982; Langerhans 2008). Although our predictions about the direction of trait change are grounded in biomechanical theory, these particular phenotypes are not always favored in low or high flow environments. For example, there is a negative relationship between body depth and hydrodynamic drag in *Lepomis macrochirus* such that deeper bodied individuals have lower drag and are favored over terete conspecifics in high flow environments (Schaefer et al. 1999). Thus, the increase in drag that is usually associated with streamlined bodies may cause the evolution of shorter, fusiform bodies and higher aspect ratio caudal fins in reservoir populations of some species.

Three of the species, *H. placitus*, *M. storeriana*, and *N. potteri*, prefer riverine environments and are largely collected only near the inflow of the Red and Washita Rivers in Lake Texoma (Figure S3). Because these species may not inhabit the reservoir

lacustrine zone, their only self-sustaining populations may be in the riverine and transitional zones with intermediate flow (Thornton et al. 1990). Occasional droughts or floods may influence the magnitude of flow in the riverine and transitional zones and ultimately displace fish from these areas (Matthews 1984), but it seems likely these species evolved phenotypes optimal for survival in these areas because evolution occurred immediately after impoundment for traits in these species. Interestingly, morphological change did not conform to *a priori* expectations for any of the three locomotive traits in *H. placitus* and *M. storeriana* and did so for two traits (body size and caudal fin shape) in *N. potteri*. Body shape was more streamlined in reservoir populations of these three species whereas body depth of all five lacustrine species increased, as predicted. The direction of morphological change in these species could be due to selective pressures associated with the riverine and transitional zones such as limited food availability, intense predation as stream predators (e.g., *Lepomis*; *Micropterus*) enter the reservoir, or local variation in flow at the bottom of the water column (e.g., the underflow; Thornton et al. 1990).

Evaluation of change in the morphological traits following stream impoundment revealed a pattern indicative of an initial bout of directional selection followed by stabilizing selection on a new adaptive peak (Cureton and Broughton 2014). Rates of evolution during the period of directional selection ranged from 0.222 to 1.0671 H and are moderate to high relative to other rates in both natural and anthropogenic systems (Hendry and Kinnison 1999; Hendry et al. 2008). It is interesting to note that the two highest rates of evolution were documented in two species that are frequently invasive outside of their range (*C. lutrensis*: 1.06 H; *C. carpio*: 1.00 H). Despite this substantial

variation in rates, traits and species evolved at the same rate following impoundment. Functionally different traits (e.g., morphological, physiological, etc.) evolve at different rates indicating that traits with similar functions evolve at relatively similar rates (Bone and Farres 2001). Because the traits in our study all contribute to swimming performance, the intensity of selection on these traits may have been similar following impoundment resulting in similar rates of evolution (Domenici and Blake 1997). This is unlikely, however, because selection was more intense on body size than body shape or caudal fin shape (Figure S4; estimated using the intensity of selection coefficient (i) from Perez and Munch 2010). The intensity of selection was similar among all species suggesting that similar rates of evolution among species may be due to equally intense selection (Figure S5). Alternatively, the additive genetic variation for each trait or species may have been exhausted during the period of directional selection such that all traits and species evolved at a similar, maximum rate (Albert et al. 2008). Because all of the species in this study share a recent evolutionary history, the amount of genetic variation underlying these traits may be relatively similar among all species limiting the potential for some traits or species to evolve faster than others.

We compared rates of evolution for three locomotive traits from eight species using a robust experimental design: all rates were estimated from the same population over the same time span. Traits did not diverge in a consistent manner potentially as a result of where each species resides in the reservoir or species-specific hydrodynamics. Traits and species evolved at the same rate, a pattern that is unlikely due to equal intensity selection on all traits and species. Rather, selection may have acted on a similar amount of additive genetic variation that underlies each of these traits in all

eight species. Directional selection on that genetic variation following the change from a stream to a reservoir may have resulted in all species rapidly evolving phenotypes that are optimal for survival in the reservoir habitat. Stabilizing selection has since maintained the reservoir phenotype on a new adaptive peak (Estes and Arnold 2007). Collectively, these results suggest that the direction of phenotypic divergence is not consistent among traits and species following a change in the environmental conditions. However, rates of evolution of the traits, regardless of the direction of change, were consistent among species. These results highlight the ability of species to evolve at similar rates and provide the impetus to understand why rates of evolution are constant among traits and species.

REFERENCES

- Adams DC, Otárola-Castillo E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**, 393-399. (doi: 10.1111/2041-210X.12035)
- Albert YA, Sawaya S, Vines TH, Knecht AK, Miller CT, Summers BR, Balabhadra S, Kingsley DM, Schluter D. 2008 The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evol.* **62**, 76–85. (doi:10.1111/j.1558-5646.2007.00259.x)
- Becker GC. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.
- Bone E, Farres A. 2001. Trends and rates of microevolution in plants. *Genetica* **112-113**, 165-182. (doi: 10.1023/A:1013378014069)
- Cureton II JC, Broughton RE. 2014. Rapid morphological divergence of a stream fish in response to changes in water flow. *Biol. Lett.* **10**. (doi: 10.1098/rsbl.2014.0352)

- Domenici P, Blake RW. 1997. The kinematics and performance of fish fast-start swimming. *J. Exper. Biol.* **200**, 1165-1179.
- Estes S, Arnold SJ. 2007 Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *Am. Nat.* **169**, 227–244. (doi:10.1086/510633)
- Franssen NR. 2011. Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evol. Appl.* **4**, 791-804. (doi: 10.1111/j.1752-4571.2011.00200.x)
- Franssen NR, Harris J, Clark SR, Schaefer JK, Stewart LK. 2013. Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proceed. Royal Soc. B* **280**. (doi: 10.1098/rspb.2012.2715)
- Gingerich PD. 1983. Rates of evolution: effects of time and temporal scaling. *Sci.* **222**, 59-161. (doi: 10.1126/science.222.24620.159-a)
- Haas TC, Blum MJ, Heins DC. 2010. Morphological responses of a stream fish to water impoundment. *Biol. Lett.* **6**, 803-806. (doi: 10.1098/rsbl.2010.0401)
- Hendry AP, Kinnison MT. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evol.* **53**, 1637-1653.
- Hendry AP, Farrugia TJ, Kinnison MT. 2008. Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.* **17**, 20-29. (doi: 10.1111/j.1365-294X.2007.03428.x)
- Jackson DA. 1993. Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches. *Ecol.* **74**, 2204-2214. (doi: dx.doi.org/10.2307/1939574)

- Jenkins RE, Burkhead NM. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda.
- Lande R. 1976. Natural selection and random genetic drift in phenotypic evolution. *Evol.* **30**, 314-334.
- Langerhans RB. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Compar. Biol.* **48**, 750-768. (doi: 10.1093/icb/icn092)
- Matthews JW. 1984. Influence of turbid inflows on vertical distribution of larval shad and freshwater drum. *Trans. Amer. Fish. Soc.* **113**, 192-198.
- Mayden R. 1991. Cyprinids of the new world. In: Cyprinid Fishes. Eds: Wingfield II, Nelson JS. Springer Netherlands. Pp. 240-263.
- Perez KO, Munch SB. 2010. Extreme selection on size in the early lives of fish. *Evol.* **64**:2450-2457. (doi:10.1111/j.1558-5646.2010.00994.x)
- Quinn TP, Adams DJ. 1996. Environmental changes affecting the migratory timing of American shad and sockeye salmon. *Ecol.* **77**, 1151-1162.
- Reznick DN, Shaw FH, Rodd FH, Shaw RG. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Sci.* **275**, 1937-1937. (doi: 10.1126/science.275.5308.1934)
- Robinson HW and Buchanan TM. 1992. Fishes of Arkansas. University of Arkansas press, Fayetteville.
- Riggs DC, Bonn EW. 1959. An annotated list of the fishes of Lake Texoma, Oklahoma and Texas. *Southwest. Nat.* **4**, 157-168
- Rohlf FJ. 2010. tpsDIG2. Stony Brook University, New York, NY. (<http://life.bio.sunysb.edu/morph/soft-dataacq.html>).

- Sambilay VC. 1990. Interrelationships between swimming speed, caudal fin aspect ratio and body length of fishes. *Fishbyte* **689**, 16-20.
- Schaefer JF, Lutterschmidt WI, Hill LG. 1999. Physiological performance and stream microhabitat use by the centrarchids *Lepomis megalotis* and *Lepomis macrochirus*. *Environ. Biol. Fish.* **54**, 303-312.
- Scott WB, Crossman EJ. 1979 Freshwater Fishes of Canada. Gordon Soules Book Pub, Vancouver.
- Siepielski AM, DiBattista JD, Carlson SM. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecol. Lett.* **12**, 1261-1276. (doi: 10.1111/j.1461-0248.2009.01381.x)
- Stockwell CA, Hendry AP, Kinnison MT. 2003. Contemporary evolution meets conservation biology. *TRENDS Ecol. Evol.* **18**, 94-101. (doi: dx.doi.org/10.1016/s0169-5347(02)00044-7)
- Thornton KW, Kimmel BL, Payne FE. 1990. Reservoir Limnology: Ecological Perspectives. John Wiley & Sons, Inc.
- Webb PW. 1982. Locomotor patterns in the evolution of Actinopterygian fishes. *Amer. Zool.* **22**:329-342. (doi: 10.1093/icb/22.2.329)
- Webb PW, De Buffrénil V. 1990. Locomotion in the biology of large aquatic vertebrates. *Transact. Amer. Fish. Soc.* **119**, 629-641. (doi: 10.1577/1548-8659(1990)119<0629:LITBOL>2.3.CO;2)
- Zelditch ML, Swiderski DL, Sheets DH, Fink WL. 2004. *Geometric morphometrics for biologists*. Academic Press, San Diego.

FIGURE 3

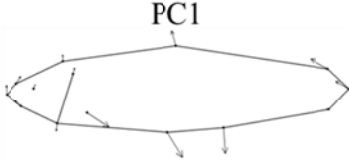

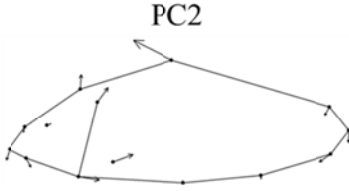
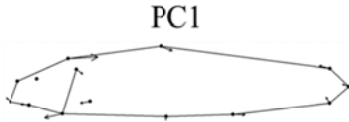
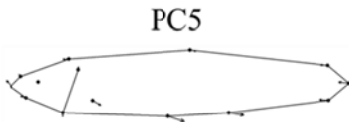
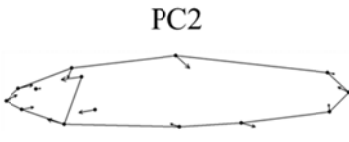

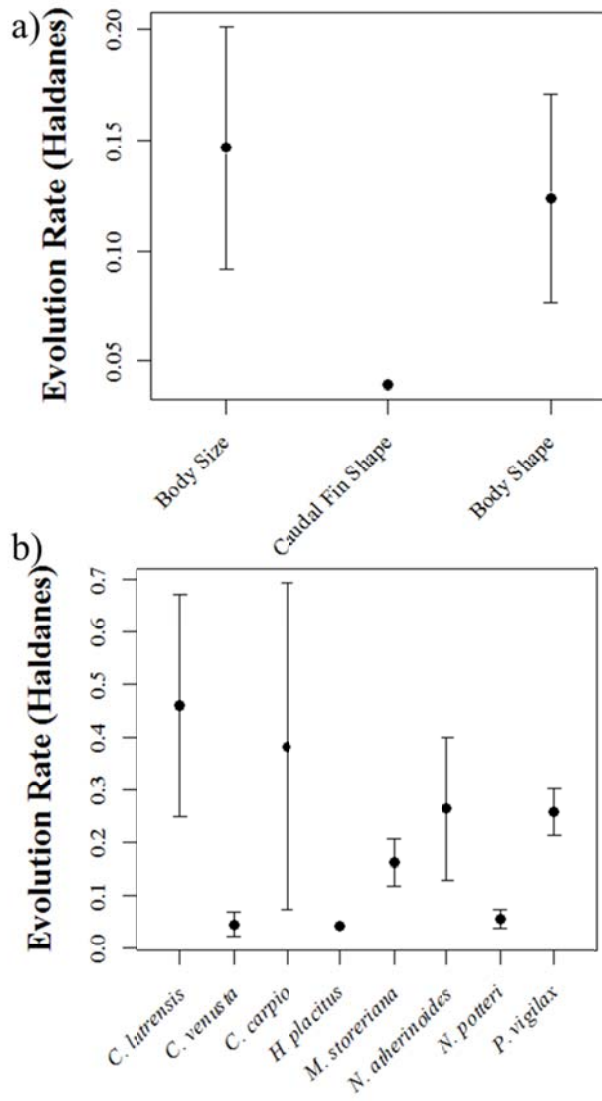
Species	Body Size	Body Shape	Caudal Fin Shape
<i>C. lutrensis</i>	↓	PC1 	↑
<i>C. venusta</i>	no change	PC1 	↑
<i>C. carpio</i>	no change	PC2 	no change
<i>H. placitus</i>	↓	no change	no change
<i>M. storeriana</i>	↓	PC1 	no change
<i>N. atherinoides</i>	↓	PC5 	no change
<i>N. potteri</i>	↑	PC2 	↓
<i>P. vigilax</i>	no change	PC1 	↑

FIGURE 4



**CHAPTER 3: INCOMPLETE CONVERGENCE OF BENTHIC BODY SHAPES
FOLLOWING PELAGIC-BENTHIC TRANSITIONS IN NORTH AMERICAN
CYPRINIDS³**

³Formatted for submission to *Evolution*

ABSTRACT

Habitat transitions are frequent drivers of phenotypic convergence in fishes. The magnitude of convergence among evolutionary lineages, however, may be constrained by lineage-specific factors. Here, we examine whether transitions from pelagic-to-benthic habitats resulted in convergent evolution of body shape in North American cyprinids. We built a phylogenetic hypothesis of 269 species of cyprinids using two nuclear and two mitochondrial genes, reconstructed the evolution of and transition between benthic and pelagic habitats, and evaluated the direction and magnitude of body shape evolution during habitat transitions. The ancestral habitat type of North American cyprinids was recovered as pelagic and followed by approximately 25 habitat transitions with pelagic-to-benthic transitions being more common. Consistent with theoretical expectations, benthic species had deeper bodies and larger heads than pelagic species although benthic and pelagic morphospaces overlapped substantially. These data suggest incomplete convergence of body shape in benthic species potentially as a result of preference in habitat type (e.g., river size). Conversely, incomplete convergence could be due to the unique evolutionary history of the benthic clades suggesting that the evolution toward benthic living is strongly constrained by lineage-specific factors.

INTRODUCTION

The evolution of similar phenotypes among divergent lineages that occupy similar environments illustrates the power of natural selection to drive convergent evolution at the cellular, molecular, and phenotypic levels (Hubbs 1941; Masters et al. 1996; Pupo et al. 2000; Feil and Burger 2007; Muschick et al. 2012). Examples from vertebrates include the evolution of intelligence in corvid bird and primate lineages (Emery and Clayton 2004), blood antifreeze glycoproteins in Arctic cod and notothenoid fishes (Chen et al. 1997), and body, leg, and tail shapes associated with in perch diameter in *Anolis* lizards (Losos et al. 1998). Although selection may drive the evolution of similar phenotypes in different groups, lineage-specific factors such as the genetic and developmental background, phenotypic modularity, and functional trade-offs, may limit the degree of morphological convergence among lineages (Hulsey and Wainwright 2002; Langerhans et al. 2006).

Stayton (2006) suggested that convergent evolution can be inferred when the convergent descendants occupy a smaller, distinct region of morphospace from their ancestors that experienced a different selective regime. However, convergent evolution may result in a pattern such that the descendant species do not occupy a morphospace completely distinct from that of their ancestors, a pattern termed incomplete convergence (Herrel et al. 2004; Stayton 2006). In some lineages, many phenotypes can achieve the same function, a pattern termed many-to-one mapping (Huley and Wainwright 2002; Wainwright et al. 2005). When multiple phenotypes can achieve the same function, descendant populations may exhibit increased phenotypic variation and occupy a larger, more diverse morphospace than that of their ancestors (Hulsey and

Wainwright 2002). This pattern, known as imperfect convergence, was recently documented in the evolution of durophagy in Moray Eels (Collar et al. 2014).

Transitions between benthic and pelagic habitats are thought to be an important driver of life-history, foraging, and locomotive diversification in freshwater fishes (Hubbs 1941; Schluter and McPhail 1992; Baker et al 2005; Svanbäck et al. 2008). Benthic fishes tend to produce fewer, larger eggs, forage on macroinvertebrates and have deeper bodies, lower aspect ratio caudal fins, and flexible bodies (Webb 1978, 1982, 1984). Pelagic fishes produce larger clutches of smaller eggs, feed on zooplankton, and use their stiff, streamlined bodies and higher aspect ratio caudal fins to swim in the water column (Webb 1978, 1982, 1984). Selection for living in these two divergent environments also results in divergence in swimming ability with deeper bodied benthic species being better unsteady, or burst, swimmers than their pelagic counterparts. Divergence of these phenotypes has been widely documented on a micro-evolutionary scale (Schluter and McPhail 1992; Robinson and Wilson 1996). Previous studies have suggested that benthic and pelagic habitats have resulted in the evolution of species with phenotypes optimized to living in each of these particular habitats (Douglas and Matthews 1992). However, the importance of these shifts in driving macroevolutionary diversification among freshwater fishes is largely unknown.

North American cyprinids are the most diverse family of North American freshwater fishes (Cyprinidae), comprising at least 325 species distributed across most of North America (Eschemeyer 2015). North American cyprinids inhabit both benthic and pelagic habitats and shifts between benthic and pelagic habitats are thought to have played an important role in the early diversification of the open posterior myodome

(OPM) clade of North American cyprinids (Hollingsworth et al. 2013). Indeed, a transition from a benthic to pelagic habitats is associated with an increased rate of lineage diversification in the OPM clade (Simons and Mayden 1999; Hollingsworth et al. 2013). Phenotypic diversification may have occurred on an even more fine scale as some cyprinids partition vertical variation in the water column (Baker and Ross 1981; Surat et al. 1982; Gorman 1988a; Gorman 1988b). Because benthic-pelagic transitions were likely important to the diversification of this group, body shape may be a trait that experienced convergent evolution following these transitions (Douglas and Matthews 1992). Indeed, selection for specific locomotive phenotypes has resulted in convergent evolution of body shape across divergent lineages including ichthyosaurs, whales, pelagic fishes, and lamnid sharks (Donley et al. 2004). We evaluated the evolution of body shape in North American cyprinids and tested the hypothesis that transitions between benthic and pelagic habitats resulted in convergent evolution of body shapes. Specifically, we predicted that benthic species would evolve larger heads, deeper bodies, and deeper caudal peduncles than pelagic species. Our results show that transition between pelagic and benthic habitats resulted in incomplete convergent evolution of body shape such that species evolved benthic body shapes following a shift to benthic habitats, but the magnitude of change in body shape depended on the unique evolutionary history of each species. These results highlight the potential for lineage-specific factors to constrain the ability of convergent evolution to produce similar phenotypes.

METHODS

Reconstructing the Evolutionary History of Pelagic-Benthic Transitions

We generated a phylogenetic hypothesis for 269 species of North American cyprinids using four genes downloaded from National Center for Biotechnology Information's GenBank database (Table S5). We downloaded sequences for all species for which they were available for cytochrome oxidase I (COI), cytochrome B (CYTB), recombination activating gene 1 (RAG1), and an intron of S7 (S7) (Table S5). We used a smaller portion of each gene that was available for most species resulting in a total alignment of 4,503 base pairs (COI [652 bp], CYTB [1141 bp], RAG1 [1520 bp], and S7 [1,190 bp]). For each gene, we aligned all available sequences using the Muscle algorithm as implemented in Geneious R2 (Edgar 2004; Kearse et al. 2012). All gene alignments were then concatenated into a single alignment for phylogenetic analysis and divided into 10 partitions: COI, CYTB, and RAG1 by codon position and the S-7 intron.

First, we analyzed the partitioned dataset using a maximum likelihood framework as implemented in RAxML BlackBox (Stamatakis et al. 2008). We used the GTRCAT model of nucleotide evolution and bootstrapped the dataset 100 times. Then, we analyzed the partitioned alignment using a Bayesian framework (Mr. Bayes v.1.8; Ronquist and Huelsenbeck 2003) as implemented on the CIPRES Science Gateway (Miller et al. 2010). We implemented the GTR + I + Γ model of nucleotide evolution and ran this analysis for 20,000,000 generations (8 chains), resampling every 1,000 generations. We discarded the first 25% of the trees generated as burn-in (5,000 trees); thus, the final topology and posterior probabilities were generated from 15,000 trees. The final tree was rooted using the common carp (*Cyprinus carpio*) which was removed for subsequent analyses.

For each species for which we had morphometric data (N=201 species), we searched the published literature to assign a species as benthic or pelagic using the criteria outlined in Hollingsworth et al. (2013): ventrally located mouth, presence of barbels, presence of a spiraled gut, build benthic nests, or feeds on primarily benthic items (Table S5). We coded species as only “benthic” or “pelagic” rather than quantifying the degree of “benthic” or “pelagic” for each species (e.g., based on the number of criteria satisfied or their vertical location in the water column) because information on all of these traits is not available for most species. We reconstructed habitat type (benthic or pelagic) using stochastic character mapping as implemented in the `make.simmap` function in the R package `phytools` (Huelsenbeck et al. 2003; Revell 2012). We used fixed priors for transition rates between habitat types and sampled 1,000 character maps. We calculated Bayesian posterior probabilities using the `describe.simmap` function in the `phytools` package (Revell 2012).

Assessing Convergent Phenotypic Evolution

We photographed the left lateral side of up to 5 specimens of each of 216 species of North American cyprinids (N=1,045 total specimens) from the Sam Noble Museum of Natural History and the Tulane University Biodiversity Research Institute (Table S5). Fifteen species were removed because the four genes used in this study were unavailable for these species (Table S5). We assigned 12 homologous landmarks to each photograph using *TPSDig2* and removed landmark variation due to translation, scaling, and rotation using a general Procrustes analysis from the “geomorph” package in R (Zelditch et al. 2004; Rohlf 2010; Adams and Otárola-Castillo 2013). We reduced the landmarks into fewer informative variables using a phylogenetically controlled

principal components (PC) analysis as implemented in the `phyl.pca` function in the `phytools` package (Revell 2012). We determined the number of informative PC using the broken-stick model (Jackson 1993). We compared each informative PC between habitat types after accounting for evolutionary history using simulation-based phylogenetic ANOVAs as implemented in the `phylANOVA` function in the `phytools` package (Garland et al. 1993; Revell 2012). We included centroid size as a covariate in all analyses. Only PC that were significantly different between habitat types were used in subsequent analyses.

We then tested for multiple lines of evidence of convergent evolution using the PC that were different between habitat types. First, we compared morphological disparity between benthic descendants and their pelagic ancestors to determine if the benthic species occupy a smaller, distinct morphospace (Stayton 2006). To estimate morphological disparity in the pelagic ancestors, we first reconstructed ancestral PC scores using maximum likelihood, assuming equal rates of transitions between habitat types. We quantified disparity as the variance in PC scores of the first pelagic ancestor of the benthic species (Collar et al. 2014). We quantified disparity among the benthic species as the variance among species' PC scores. Because multiple benthic species evolved from a single pelagic ancestor, comparison of all benthic descendants to pelagic ancestors would result in substantially different sample sizes for the two groups. As such, we performed 100 bootstrapped replicates of PC scores for the benthic descendants and compared variance in each of these replicates with that of the ancestors using Levene's heterogeneity of variance test. We report the average p-value for the 100 bootstrap replicates.

Higher disparity in the morphospace of benthic descendants does not rule out convergent evolution if benthic taxa are diverging in the same direction from their ancestors. To evaluate the direction of evolution in benthic taxa, we compared PC scores from each clade of convergent benthic taxa to the pelagic species to which they are most closely related. We considered each clade of convergent benthic taxa and their most closely sister pelagic species as a monophyletic “phylogenetic pair.” We compared PC scores between habitat types and among “phylogenetic pairs” (and their interaction) using an ANCOVA. We included body size as a covariate. Post-hoc analyses of “phylogenetic pairs” were carried out using Tukey Honest Significant Differences tests. All analyses were performed using R v.3.1.1 Statistical Software (R Core Team; Vienna, Austria).

RESULTS

Evolutionary History of Pelagic-Benthic Transitions

Maximum likelihood and Bayesian analyses converged on a relatively similar topology with the exception of *i*) relationships among the four major clades described below and *ii*) the placement of *Notemigonus crysoleucas* (Figures S6, S7). Therefore, we focus our discussion and subsequent analyses on the Bayesian topology (Figure S7). We recovered strong support for previously described “creek chub”, “plagopterin”, “western”, and “OPM” clades with only *N. crysoleucas* recovered outside of these clades. Support was high for all relationships within the “creek chub” clade and most relationships within the “plagopterin” clade with the exception of the node identifying the sister group to *Couesius plumbeus* and *Margariscus margarita*. All nodes within the “western” clade, with the exception of two nodes within *Gila*, also received high

support. Within the “OPM” clade, support was high for most clades with the notable exception of the unresolved clade that includes the majority of *Notropis* species. Within this “*Notropis*” clade, *Ericymba*, *Hybopsis*, *Lythrurus*, and *Hybognathus* were recovered as monophyletic. Several other genera, including *Luxilus*, *Tampichthys*, *Cyprinella*, *Pimephales*, and *Pteronotropis*, were monophyletic with one or two exceptions, potentially a result of missing sequence data for some of these species. We recovered several strongly supported clades of *Notropis* although relationships among *Notropis* clades were not always strongly supported.

To insure our results were not biased based on topology choice, we estimated the number of habitat transitions on likelihood and Bayesian phylogenetic hypotheses. Because the number and location of transitions was almost identical, we discuss only the results from reconstruction of habitat on the Bayesian hypothesis (Figure S8). A total of 24.9 habitat transitions were estimated across 1,000 trees with 7.8 transitions from benthic to pelagic habitats and 17.1 transitions occurring from pelagic to benthic habitats (Figure 5). The ancestral habitat state for all North American cyprinids was recovered as pelagic. There was an early habitat shift to benthic habitats in the “creek chub” and “plagopterin” clades. The majority of the “western” clade was recovered as pelagic although there were a few transitions to benthic habitats in this clade (Figure 5). The ancestral state of the “OPM” clade was pelagic although there was a transition to benthic environments early in the diversification of this clade. Subsequent diversification within the OPM clade occurred along a benthic axis until the unresolved *Notropis* clade at which point there was a transition back to pelagic habitats. Most of the transitions from pelagic to benthic habitats occurred in the unresolved *Notropis* clade.

Although incomplete resolution of the relationships in this clade prevented a more precise estimate of the number of habitat transitions, there were a minimum of nine pelagic-to-benthic transitions in this clade because up to three transitions could collapse into a single clade upon resolution of this group

Convergent Evolution of Benthic Taxa

Broken-stick analysis of the 24 PC revealed only the first four to be informative. The first four PC explained 16.3%, 11.8%, 10.4%, and 8.5% of the variation in body shape respectively, and 47%, cumulatively. PC1 accounted for variation in head size and shape as well as body depth and the location of the insertion of the dorsal, pelvic, and anal fins (Figure 6). Variation in PC2 reflected variation in body shape with the primary changes in the location of the dorsal fin relative to the pelvic and anal fins (Figure 6). Variation in PC3 accounted for changes in head shape and orientation as well as variation along the ventral side of the fish and the orientation of the caudal peduncle (Figure 6). PC4 accounted for variation along the vertical axis of the fish (i.e., head and body depth; Figure 6). When we accounted for phylogenetic relationships, only PC2 ($F=64.890$, $P=0.001$) and PC4 ($F=99.541$, $P=0.001$) were different between habitat types suggesting that variation in PC1 and PC3 may be due to evolutionary history rather than habitat differences. PC2 scores were higher for benthic species indicating pointed snouts, deeper bodies, and anterior shifted dorsal fins (Figure 7). PC4 scores were significantly lower for benthic species indicating they had a larger head and deeper body than pelagic species (Figure 7).

We identified nine “phylogenetic pairs” of convergent benthic species and their most-closely related pelagic species for additional analyses. The morphospace of

benthic descendants was not significantly smaller than that of their most recent common pelagic ancestors within these “phylogenetic pairs” (PC2: $P=0.158$; PC4: $P=0.141$; Figure 8). Within these “phylogenetic pairs,” benthic species had significantly higher PC2 scores than their pelagic counterparts ($F_{1,316}=16.047$, $P<0.001$, $\eta^2=0.048$; Figure 7). Nonetheless, there was substantial overlap in PC2 scores between benthic and pelagic species. The interaction between habitat type and phylogenetic pair was significant for PC4 indicating that the effect of habitat type on PC4 scores depends on each phylogenetic pair ($F_{1,316}=8.763$, $P=0.004$, $\eta^2=0.027$; Figure 7). Post-hoc analysis of PC4 revealed that benthic species had significantly lower scores than pelagic species for phylogenetic pairs 1, 4, 6, and 9 (Table S5). The benthic species at node 8 had significantly higher PC4 scores than the pelagic species and PC4 scores did not differ between habitats for the other 4 species pairs (Table S5). Although benthic species occupy a statistically different morphospace from pelagic species on PC2 and, in some cases, PC4, there was sufficient overlap between in body shape between habitat types suggesting incomplete convergent evolution of body shape (Figure 8).

DISCUSSION

We expected transitions from pelagic to benthic habitats to result in the evolution of similar body shapes in benthic species of North American cyprinids. First, we estimated the frequency of habitat transitions using a phylogenetic hypothesis built using a super-matrix approach (de Queiroz and Gatesy 2007). Using this approach, we recovered several relationships consistent with previously published studies including a “creek chub” clade, “western” clade, a plagioplerin clade, and an “OPM” clade (Simons and Mayden 1997; Simons and Mayden 1999; Simons et al. 2003; Schönhuth et al.

2012). Support within most of these clades was strong (with a few exceptions on the maximum likelihood topology), with the exception of the clade containing the majority of the *Notropis* species in the “OPM” clade. Within this large, unresolved clade are several genera that were recovered as monophyletic and several more that were monophyletic with the exception of one or two species (e.g., *Pimephales*). The polyphyly of these genera is likely a result of missing data for these species (e.g., *Tampichthys*; Schönhuth et al. 2008). Several groups of *Notropis* species were strongly supported although these clades did not always correspond to *Notropis* subgenera (e.g., *Hydrophlox*; Cashner et al. 2011). Despite missing data complicating more fine scale resolution of phylogenetic relationships, habitat transitions were largely resolved although two of the transitions could be collapsed depending on resolution of the *Notropis* clade. Interestingly, pelagic-to-benthic transitions occurred more frequently than benthic-to-pelagic transitions. The diversification of North American cyprinids along a benthic axis prior to the pelagic *Notropis* radiation may have limited available habitat for subsequent pelagic-to-benthic transitions.

The transition from a benthic to pelagic habitat associated within the unresolved *Notropis* clade is associated with an increased rate of lineage diversification (Hollingsworth et al. 2013). We expected that transitions back to benthic habitats within this pelagic clade would result in larger heads, deeper bodies, and deeper caudal peduncles in benthic species. These locomotive phenotypes optimize unsteady swimming ability which should be selected for in benthic environments (Webb 1982). PC2 and PC4, which could not be explained only by phylogenetic relationships, consistently diverged between benthic and pelagic species. Analysis of these two PC

indicated morphological changes consistent with biomechanical theory: pelagic species had smaller heads with pointed snouts, streamlined bodies, posterior shifted dorsal fins, and anterior shifted pelvic and anal fins relative to benthic species (Webb 1978, 1982). Although not enough data are available to robustly assess whether vertical stratification of the water column resulted in a more fine level of phenotypic diversification, there is an inverse relationship between PC2 and vertical depth for two independent data sets suggesting this hypothesis may be plausible (Baker and Ross 1981; Surat et al. 1982; Gorman 1988a; Gorman 1988b); thus, species that lived lower in the water column had more benthic phenotypes, as predicted by this hypothesis. Although support for this hypothesis would suggest phenotypic diversification on a very fine spatial scale, our data clearly show that selection for living in benthic and pelagic habitats is strong enough to drive evolution of specific body shapes in North American cyprinids.

Stayton (2006) defined explicit criteria for detecting convergent evolution, suggesting that descendant species should occupy a smaller, but distinct morphospace from their ancestors that experienced a different selection regime. Accordingly, convergent evolution should have resulted in a smaller, distinct morphospace in benthic species relative to their pelagic ancestors. Although PC2 and PC4 scores were significantly different between benthic and pelagic species, there was sufficient overlap in the morphospace of benthic and pelagic species suggesting that they do not occupy distinct morphospaces. Further, benthic species did not exhibit reduced morphological disparity relative to their pelagic ancestors; thus, it convergence of benthic phenotypes in North American cyprinids is incomplete (Herrel et al. 2004). There are several mutually non-exclusive factors that may have resulted in this pattern. First, our

dichotomous classification of “benthic” and “pelagic” species may be too broad relative to the degree of habitat partitioning in streams. Indeed, data suggest that phenotypic diversification may have occurred as a result of partitioning the water column. However, more robust data are needed to evaluate this hypothesis. Second, we did not consider variation in micro- or macro-habitats among species. For example, the magnitude of convergence may be different in species that live in habitats in different flow regimes such that benthic species in large rivers have a more pelagic body shape than benthic species in lakes (Langerhans 2008). Third, the benthic phenotypes in the “creek chub” and “plagopterin” clades may have been optimized for survival in those lineages whereas “OPM” minnows may have a different optimal benthic phenotype. Fourth, the evolution of benthic phenotypes may be constrained by the evolutionary history of any particular lineage (Langerhans et al. 2006). The benthic phenotypes in the “OPM” clade may be unable to evolve in the “creek chub”, “plagopterin”, or “western” clades due to lineage-specific evolutionary constraints. More specifically, the benthic “western” species have a more benthic body shape than pelagic “western” species, but do not closely resemble the body shape of “OPM” benthic species perhaps due to developmental or genomic constraints.

Habitat transitions are important drivers of phenotypic diversification because of selection for specific locomotive demands in divergent environments. Such environmental demands have led to the convergent evolution of “thunniform” body shape in ichthyosaurs, whales, pelagic fishes, and lamnid sharks (Donley et al. 2004). We demonstrated incomplete convergent evolution of body shape in benthic species of North American minnows. Benthic species evolved larger heads, deeper bodies, and

deeper caudal peduncles than pelagic species. This suggests divergent selection drove the evolution of habitat-specific phenotypes that are optimal for survival in benthic and pelagic environments. However, benthic species did not occupy a morphospace distinct from that of pelagic species leading us to conclude that convergent evolution of body shape in North American cyprinids is incomplete – i.e., benthic species tend to have deeper bodies than pelagic species, but the degree of body shape convergence in benthic taxa dependent on the specific clade. Incomplete convergence of body shape could be due to several mutually non-exclusive factors including habitat preferences of benthic species as well as lineage-specific factors. These results highlight the potential for evolution to drive convergence of body shape despite strong constraints among benthic lineages.

REFERENCES

- Adams, D. C., and E. Otárola-Castillo. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* 4: 393-399.
- Baker, J. A., W. A. Cresko, S. A. Foster, and D. C. Heins. 2005. Life-history differentiation of benthic and limnetic ecotypes in a polytypic population of threespine stickleback (*Gasterosteus aculeatus*). *Evol. Ecol. Res.* 7: 121-131.
- Cashner, M. F., K. R. Piller, and H. L. Bart. 2011. Phylogenetic relationships of the North American cyprinid subgenus *Hydrophlox*. *Mol. Phylogenet. Evol.* 59: 725-735.

- Chen, L., A. L. DeVries, and C.-H. C. Cheng. 1997. Convergent evolution of antifreeze glycoproteins in Antarctic notothenoid fish and Arctic cod. *Proceed. Nat. Acad. Sci. U.S.A.* 94: 3817-3822.
- Collar, D. C., J. S. Reece, M. E. Alfaro, P. C. Wainwright, and R. S. Mehta. 2014. Imperfect morphological convergence: variable changes in cranial structures underlie transitions to durophagy in Moray Eels. *Amer. Nat.* 183: E168-184.
- de Queiroz, A., and J. Gatesy. 2007. The supermatrix approach to systematics. *TRENDS Ecol. Evol.* 22: 34-41.
- Donley, J. M., C. A. Sepulveda, P. Konstantinidis, S. Gemballa, and R. E. Shadwick. 2004. Convergent evolution in mechanical design of lamnid sharks and tunas. *Nat.* 429: 61-65.
- Douglas, M. E., and W. J. Matthews. 1992. Does morphology predict ecology? Hypothesis testing within a freshwater stream fish assemblage. *Oikos* 65: 213-224.
- Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32: 1792-1797.
- Emery, N. J., and N. S. Clayton. 2004. The mentality of crows: convergent evolution of intelligence in corvids and apes. *Sci.* 306: 1903-1907.
- Eschmeyer, W. N. (ed). *CATALOG OF FISHES: GENERA, SPECIES, REFERENCES*. Available at <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>. Accessed April 04, 2015.

- Feil, R., and F. Berger. 2007. Convergent evolution of genomic imprinting in plants and animals. *Trends Genet.* 23: 192-199.
- Garland, T., Jr, A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Systemat. Biol.* 42: 265-292.
- Gorman, O. T. 1988a. An experimental study of habitat use in an assemblage of Ozark minnows. *Ecol.* 69: 1239-1250.
- Gorman, O. T. 1988b. The dynamics of habitat use in a guild of Ozark minnows. *Ecol. Monographs* 58: 1-18.
- Herrel, A. B., B. Vanhooydonck, and R. Van. Damme. 2004. Ominivory in lacertid lizards: adaptive evolution or constraint? *J. Evol. Biol.* 17: 974-984.
- Hollingsworth, P. R., Jr., A. M. Simons, J. A. Fordyce, and C. D. Hulsey. 2013. Explosive diversification following a benthic to pelagic shift in freshwater fishes. *BMC Evol. Biol.* 13: 272-281.
- Hubbs, C. L. 1941. The relation of hydrological conditions to speciation in fishes. In: *A Symposium on Hydrobiology* (pp. 182-195). Madison, University of Wisconsin Press.
- Huelsenbeck J. P., R. Nielsen, and J. P. Bollback. 2003. Stochastic mapping of morphological characters. *Systemat. Biol.* 52:131-158.
- Hulsey, C. D., and P. C. Wainwright. 2002. Projecting mechanics into morphospace: disparity in feeding system of labrid fishes. *Proc. Royal Soc. B: Biol. Sci.* 269: 317-326.
- Jackson, D. A. 1993. Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches. *Ecol.* 74: 2204-2214.

- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and expandable desktop software platform for the organization and analysis of sequence data. *Bioinformat.* 28: 1647-1649.
- Langerhans, R. B., J. H. Knouft, and J. B. Losos. 2006. Shared and unique features of diversification in greater Antillean *Anolis* ecomorphs. *Evol.* 60: 362-369.
- Langerhans, R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comparat. Biol.* 48: 750-768.
- Losos, J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodríguez-Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Sci.* 279: 2115-2118.
- Masters, B. S. S., K. McMillan, E. A. Sheta, J. S. Nishimura, L. J. Roman, P. and Martasek. 1996. Neuronal nitric oxide synthase, a modular enzyme formed by convergent evolution: structure studies of a cysteine thiolate-liganded heme protein than hydroxylates L-arginine to produce NO as a cellular signal. *FASEB J.* 1996: 552-558.
- Miller, M. A., W. Pfeiffer, and T. Schwartz., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in *Gateway Computing Environments (GCE)*, 2010. IEEE, 2010. pp. 1-8.
- Muschick, M., A. Indermaur, and W. Salzburger. 2012. Convergent evolution within an adaptive radiation of cichlid fishes. *Curr. Biol.* 22: 2362-2368.

- Pupo, G. M., R. Lan, and P. R. Reeves. 2000. Multiple independent origins of *Shigella* clones of *Escherichia coli* and convergent evolution of many of their characteristics. *Proc. Nat. Acad. Sci. U.S.A.* 97: 10567-10572.
- Revell, L. J. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3: 217-223.
- Robinson, B. W., and D. S. Wilson. 1996. Genetic variation in phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evol. Ecol.* 10: 631-652.
- Rohlf, F. J. 2010. tpsDIG2. Stony Brook University, New York, NY.
(<http://life.bio.sunysb.edu/morph/soft-dataacq.html>).
- Ronquist, F. and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformat.* 19: 1572-1574.
- Schönhuth, S., I. Doadrio, O. Doinguez-Dominguez, D. M. Hillis, and R. L. Mayden, R. L. 2008. Molecular evolution of southern North American Cyprinidae (Actinopytergii), with description of the new genus *Tampitchthys* from central Mexico. *Mol. Phylogenet. Evol.* 47: 729-756.
- Schönhuth S., D. K. Shiozawa, T. E. Dowling, and R. L. Mayden. 2012. Molecular systematics of western North American cyprinds (Cypriniformes: Cyprinidae). *Zootaxa* 3586: 281-303.
- Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. *Amer. Nat.* 140: 85-108.

- Simons, A. M., and R. Mayden. 1997. Phylogenetic relationships of the creek chubs and the spine-fins: an enigmatic group of North American cyprinid fishes (Actinopterygii Cyprinidae). *Cladistics* 13: 187-205.
- Simons, A. M., and R. L. Mayden. 1999. Phylogenetic relationships of North American cyprinids and assessment of homology of the open posterior myodome. *Copeia* 1999: 13-21.
- Simons, A. M., P. B. Berendzen, and R. L. Mayden. 2003. Molecular systematics of North American phoxinin genera (Actinopterygii: Cyprinidae) inferred from mitochondrial 12S and 16S ribosomal RNA sequences. *Zool. J. Linnean Soc.* 139: 63-80.
- Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML Web Servers. *Systemat. Biol.* 57: 758-771.
- Stayton, C. T. 2006. Testing hypotheses of convergence with multivariate data: morphological and functional convergence among herbivorous lizards. *Evol.* 60: 824-841.
- Svanbäck, R., P. Eklöv, R. Fransson, and K. Holmgren. 2008. Intraspecific competition drives multiple species resource polymorphism in fish communities. *Oikos* 117: 114-124.
- Wainwright, P. C., M. E. Alfaro, D. I. Bolnick, and C. D. Hulsey. 2005. Many-to-one mapping of form and function: a general principle in organismal design. *Integrat. Comparat. Biol.* 45:256-262.
- Webb, P. W. 1978. Fast-start performance and body form in seven species of teleost fish. *J. Exper. Biol.* 74:211-226.

Webb, P. W. 1982. Locomotor patterns in the evolution of Actinopterygian fishes.

Amer. Zool. 22: 329-342.

Webb, P. W. 1984. Body form, locomotion and foraging in aquatic vertebrates. Amer.

Zool. 24: 107-120.

Zelditch M. L., D. L. Swiderski, D. H. Sheets, and W. L. Fink. 2004. Geometric

morphometrics for biologists. - Academic Press.

FIGURE 5

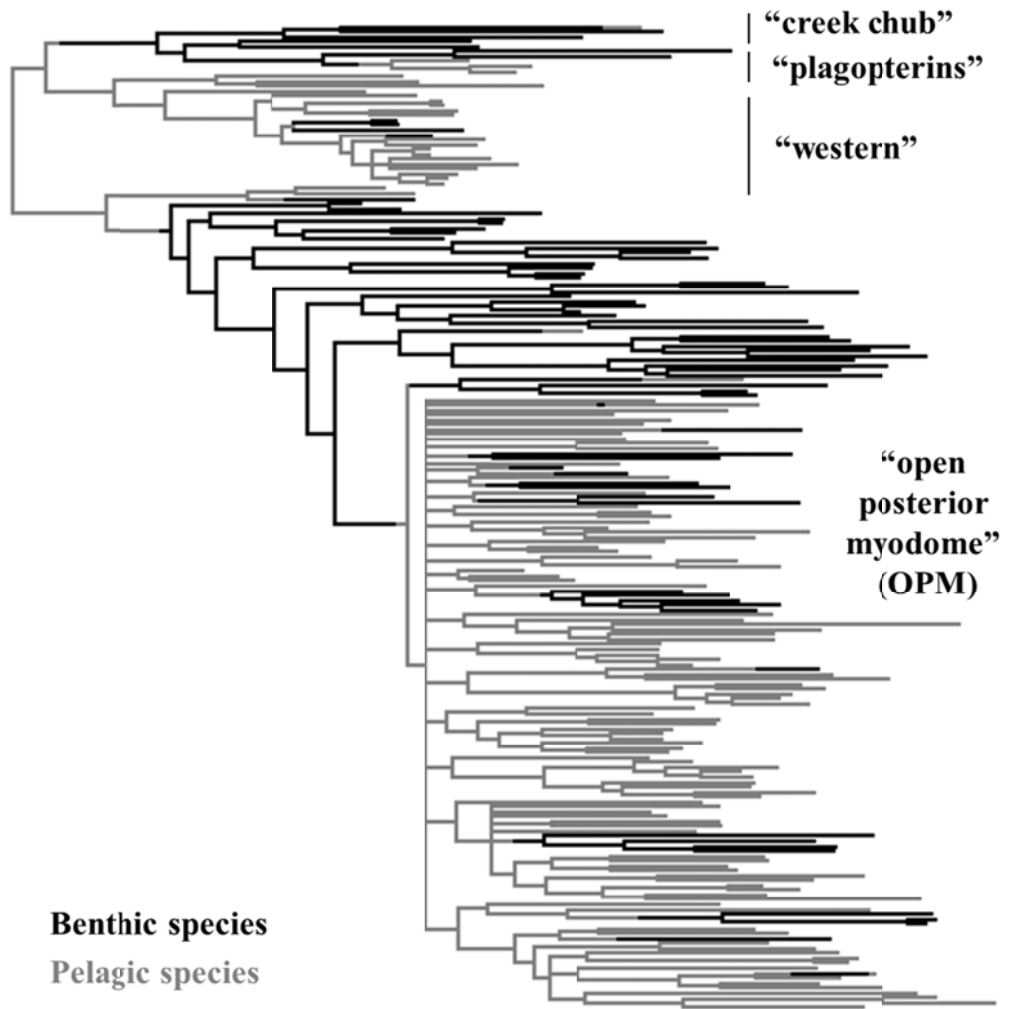


FIGURE 6

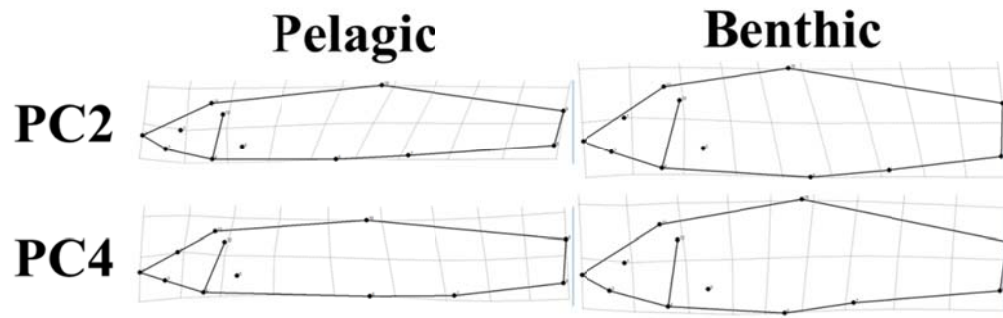


FIGURE 7

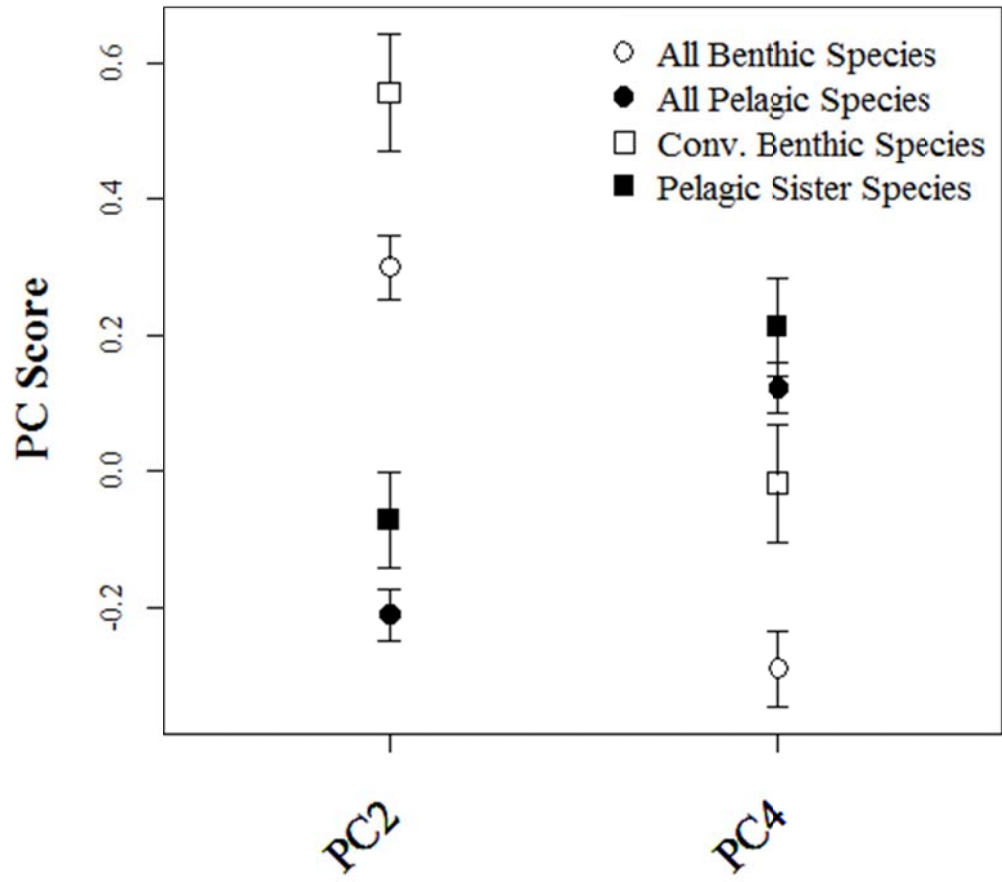
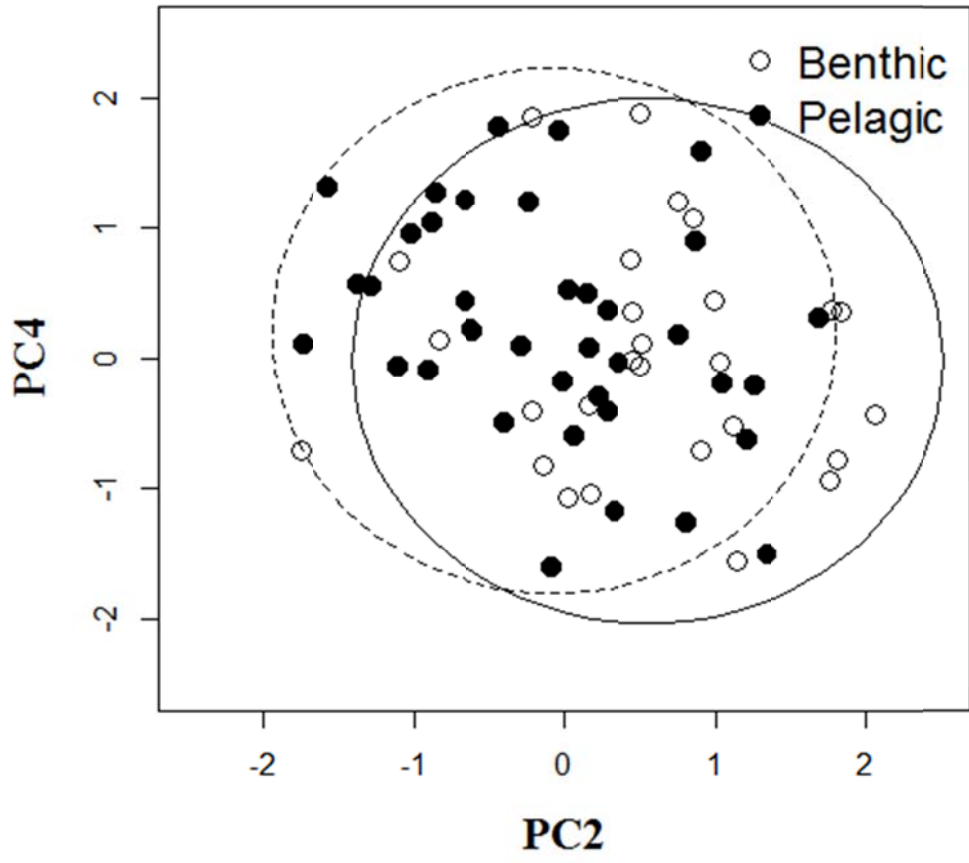


FIGURE 8



APPENDIX

TABLE S1

Species	Locality	OKMNH	Year Col	Year Imp	n	
<i>C. lutrensis</i>	Red River	33156	1963	19	3	
	Red River	61047	1999	45	16	
	Lake Texoma	30202	1949	5	2	
	Lake Texoma	29153	1951	7	19	
	Lake Texoma	27629	1954	10	20	
	Lake Texoma	30030	1958	14	11	
	Lake Texoma	31141	1959	15	10	
	Lake Texoma	32685	1960	16	12	
	Lake Texoma	39704	1962	18	16	
	Lake Texoma	39506	1963	19	20	
	Lake Texoma	39519	1964	20	19	
	Lake Texoma	50386	1993	49	8	
	Lake Texoma	62029	2000	56	20	
	Lake Texoma	65369	2001	57	20	
	<i>C. venusta</i>	Red River	46828	1990	46	22
Lake Texoma		30204	1949	5	11	
Lake Texoma		30190	1949	5	4	
Lake Texoma		29152	1951	7	2	
Lake Texoma		27628	1954	10	12	
Lake Texoma		27111	1955	11	3	
Lake Texoma		29856	1956	12	1	
Lake Texoma		37030	1957	13	1	
Lake Texoma		31119	1958	14	5	
Lake Texoma		39698	1962	18	17	
Lake Texoma		39509	1963	19	18	
Lake Texoma		39523	1964	20	2	
Lake Texoma		62988	1995	51	21	
Lake Texoma		60342	1999	55	19	
Lake Texoma		62201	2000	56	18	
Lake Texoma		65499	2001	57	20	
<i>C. carpio</i>		Red River	51199	1992	48	11
		Lake Texoma	27020	1949	5	1
	Lake Texoma	27005	1949	5	2	
	Lake Texoma	27312	1951	7	1	
	Lake Texoma	27154	1954	10	8	
	Lake Texoma	37023	1957	13	1	
	Lake Texoma	27190	1957	13	3	
	Lake Texoma	39701	1962	18	1	
	Lake Texoma	44169	1989	45	5	
	Lake Texoma	50383	1993	49	7	
	Lake Texoma	60266	1999	55	15	
	Lake Texoma	62227	2000	56	17	
	Lake Texoma	65370	2001	57	21	

<i>H. placitus</i>	Red River	31461	1952	8	19
	Lake Texoma	30250	1948	4	1
	Lake Texoma	39440	1950	6	9
	Lake Texoma	29154	1951	7	2
	Lake Texoma	29858	1956	12	7
	Lake Texoma	50384	1993	49	8
	Lake Texoma	60112	1999	55	1
	Lake Texoma	60090	1999	55	2
	Lake Texoma	62228	2000	56	9
	Lake Texoma	66786	2001	57	9
<i>M. storeriana</i>	Red River	45347	1990	46	1
	Red River	71640	2002	58	15
	Red River	77137	2005	61	5
	Lake Texoma	30247	1948	4	1
	Lake Texoma	27626	1954	10	10
	Lake Texoma	29851	1956	12	22
	Lake Texoma	37029	1957	13	16
	Lake Texoma	41617	1978	34	27
	Lake Texoma	60091	1999	55	21
	Lake Texoma	61841	2000	56	14
<i>N. atherinoides</i>	Lake Texoma	65332	2001	57	5
	Red River	60901	1999	45	12
	Red River	61105	1999	45	9
	Red River	76743	1999	45	14
	Lake Texoma	30201	1949	5	5
	Lake Texoma	27443	1954	10	1
	Lake Texoma	27579	1955	11	1
	Lake Texoma	29854	1956	12	21
	Lake Texoma	31142	1959	15	2
	Lake Texoma	36930	1959	15	4
<i>N. potteri</i>	Lake Texoma	39707	1962	18	15
	Lake Texoma	41619	1978	34	6
	Lake Texoma	50385	1993	49	3
	Lake Texoma	60113	1999	55	14
	Lake Texoma	62229	2000	56	7
	Lake Texoma	66807	2001	57	21
	Red River	33095	1963	19	8
	Lake Texoma	27627	1954	10	3
	Lake Texoma	29855	1956	12	6
	Lake Texoma	37024	1957	13	1
Lake Texoma	36936	1959	15	1	
Lake Texoma	41618	1978	34	13	
Lake Texoma	60138	1999	55	2	
Lake Texoma	61861	2000	56	20	
Lake Texoma	66843	2001	57	3	
Lake Texoma	66785	2001	57	3	

<i>P. vigilax</i>	Lake Texoma	66849	2001	57	1
	Red River	80558	2010	66	30
	Lake Texoma	39442	1950	6	3
	Lake Texoma	28108	1953	9	11
	Lake Texoma	27631	1954	10	15
	Lake Texoma	27782	1955	11	9
	Lake Texoma	30155	1956	12	9
	Lake Texoma	30031	1958	14	19
	Lake Texoma	31145	1959	15	25
	Lake Texoma	39699	1962	18	19
	Lake Texoma	39513	1963	19	17
	Lake Texoma	40615	1971	27	20
	Lake Texoma	44149	1989	45	18
	Lake Texoma	48068	1993	49	11
	Lake Texoma	63013	1995	51	23
	Lake Texoma	60315	1999	55	20
Lake Texoma	62031	2000	56	23	

TABLE S2

Species	Trait	Habitat			Temporal		
		F-value	df	P-value	F-value	df	P-value
<i>C. lutrensis</i>	Body size	25.538	1, 37	<0.001	3.668	1, 175	0.057
	Body shape	7.794	5, 32	<0.001	22.951	5, 170	<0.001
	CFAR	6.824	1, 19	0.017	14.014	1, 150	<0.001
<i>C. venusta</i>	Body size	1.576	1, 40	0.217	0.000	1, 152	0.985
	Body shape	19.372	5, 35	<0.001	15.294	5, 147	<0.001
	CFAR	11.146	1, 29	0.002	6.975	1, 26	0.014
<i>C. carpio</i>	Body size	0.215	1, 30	0.647	4.391	1, 80	0.039
	Body shape	8.645	6, 24	<0.001	10.613	6, 74	<0.001
	CFAR	2.915	1, 25	0.100	0.096	1, 61	0.757
<i>H. placitus</i>	Body size	139.850	1, 26	<0.001	41.347	1, 46	<0.001
	Body shape	1.614	3, 23	0.213	0.097	3, 43	0.961
	CFAR	1.054	1, 19	0.318	0.067	1, 37	0.798
<i>M. storeriana</i>	Body size	14.746	1, 24	<0.001	3.377	1, 114	0.069
	Body shape	0.806	7, 17	0.594	9.155	7, 107	<0.001
	CFAR	1.150	1, 5	0.333	0.280	1, 99	0.598
<i>N. atherinoides</i>	Body size	8.284	1, 54	0.006	0.099	1, 100	0.754
	Body shape	16.723	5, 49	<0.001	8.317	5, 95	<0.001
	CFAR	0.485	1, 29	0.492	0.093	1, 84	0.762
<i>N. potteri</i>	Body size	6.572	1, 13	0.024	1.163	1, 51	0.286
	Body shape	17.346	5, 8	<0.001	3.926	5, 46	0.005
	CFAR	4.973	1, 10	0.050	1.386	1, 47	0.245
<i>P. vigilax</i>	Body size	10.251	1, 51	0.002	0.914	1, 240	0.340
	Body shape	10.434	5, 46	<0.001	7.532	5, 235	<0.001
	CFAR	24.890	1, 49	<0.001	3.143	1, 210	0.078

TABLE S3

Species	Body Size		PCI		PC2		PC3		PC4		PC5		PC6		PC7		CFAR	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
<i>C. laurenzi</i>	H	5	0.3910	10	1.0671	57	0.0034	5	0.2740	14	0.1078	57	0.0022	-	-	-	57	0.0155
	SE		0.0100		0.0266		0.0060		0.0100		0.0669		0.0118					0.0098
<i>C. venusta</i>	H	57	0.0046	13	0.0695	57	0.0222	57	0.0036	57	0.0099	57	0.0022	-	-	-	57	0.0156
	SE		0.0054		0.0818		0.0116		0.0092		0.0088		0.0060					0.0154
<i>C. carpio</i>	H	57	0.0209	57	0.0266	10	1.0002	5	0.0412	57	0.0489	57	0.0390	45	0.1072	-	57	0.0189
	SE		0.0482		0.0650		0.0216		0.0322		0.0325		0.0306		0.0903			0.0495
<i>H. placinus</i>	H	49	0.0436	57	0.0001	57	0.0227	57	0.0176	-	-	-	-	-	-	-	57	0.0110
	SE		0.0129		0.0073		0.0172		0.0127		-		-		-			0.0078
<i>M. storeriana</i>	H	4	0.3448	13	0.1573	55	0.0357	4	0.0836	4	0.2160	13	0.1361	57	0.0060	57	0.0017	
	SE		0.0106		0.140		0.0200		0.0118		0.0120		0.0300		0.0134			0.0081
<i>N. atherinoides</i>	H	5	0.2722	5	0.4968	57	0.0134	57	0.0006	57	0.0036	56	0.0273	-	-	-	57	0.0034
	SE		0.0067		0.0080		0.0138		0.0150		0.0142		0.0083					0.0083
<i>N. potteri</i>	H	10	0.0912	57	0.0090	55	0.0455	10	0.0326	57	0.0087	57	0.0106	-	-	-	57	0.0301
	SE		0.0160		0.0166		0.0099		0.0224		0.0069		0.0116					0.0190
<i>P. vigilax</i>	H	57	0.0021	11	0.3433	57	0.0080	6	0.1532	12	0.3160	57	0.0045	-	-	-	14	0.2190
	SE		0.0057		0.1687		0.0091		0.0176		0.0778		0.0065					0.0613

TABLE S4

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	Total
<i>C. lutrensis</i>	20.0	17.5	11.1	9.5	6.7	-	-	64.8
<i>C. venusta</i>	21.9	15.0	12.2	9.0	6.7	-	-	64.8
<i>C. carpio</i>	23.0	16.5	12.3	9.4	7.1	6.0	-	74.3
<i>H. placitus</i>	29.2	19.7	10.4	-	-	-	-	59.3
<i>M. storeriana</i>	20.1	12.9	11.5	8.8	7.2	6.9	5.7	73.1
<i>N. atherinoides</i>	21.7	17.0	12.1	7.8	7.0	-	-	65.6
<i>N. potteri</i>	25.9	15.2	11.3	8.8	7.2	-	-	68.4
<i>P. vigilax</i>	19.0	13.2	10.7	8.5	7.2	-	-	58.6

TABLE S5

Species	Lot Nu.	N	Habitat	Clade	COI	CYTB	S7	RAG1
<i>Acrocheilus alutaceus</i>	TUBRI 121821	5	Benthic	1	EU523893	JX443090	JX443312	JX443415
<i>Agosia chrysogaster</i>	TUBRI 103388	5	Benthic	3	HQ556979	JX443014	JX443240	JX443343
<i>Algansea affinis</i>	TUBRI 10672	1	Benthic	-	-	-	-	-
<i>Algansea barbata</i>	TUBRI 31873	5	Benthic	3	-	FJ913782	FJ913823	-
<i>Algansea dugesi</i>	TUBRI 30847	1	Benthic	-	-	-	-	-
<i>Algansea popoche</i>	TUBRI 31963	5	Benthic	-	JQ935851	JX443013	JX443239	JX443342
<i>Algansea rubescens</i>	TUBRI 30911	1	Benthic	-	-	-	-	-
<i>Algansea tincella</i>	TUBRI 31866	5	Benthic	3	JQ935851	DQ324092	JX443237	JX443340
<i>Aztecula sallaei</i>	TUBRI 31866	5	Pelagic	-	JN024817	GQ249850	EU082732	EU082580
<i>Campostoma anomalum</i>	OKMNH 78530	5	Benthic	-	KF929676	DQ486282	EU082705	EF452827
<i>Campostoma oligolepis</i>	OKMNH 71881	5	Benthic	-	JN024834	JQ712314	EU082703	EU082551
<i>Campostoma ornatum</i>	TUBRI 95915	5	Benthic	-	HQ557401	JF343098	HQ608620	EU082549
<i>Campostoma paucibradii</i>	TUBRI 174210	5	Benthic	-	JN024846	DQ324065	EU082704	EU082552
<i>Chrosomus neogaeus</i>	TUBRI 114005	5	Pelagic	-	EU525073	EU755059	-	-
<i>Clinostomus elongatus</i>	TUBRI 493	5	Pelagic	2	EU524488	GU182775	GU134191	EU711112
<i>Clinostomus funduloides</i>	OKMNH 77720	5	Pelagic	2	JN024984	JX443000	JX443227	JX443329
<i>Codoma ornata</i>	TUBRI 94062	5	Pelagic	8	-	KJ755261	KJ755297	KJ755327
<i>Couesius plumbeus</i>	TUBRI 5306	5	Benthic	-	HQ579027	AY281033	GU134196	GU136297
<i>Cyprinella analostana</i>	TUBRI 27061	5	Pelagic	-	JN025171	GQ275237	-	GQ275300
<i>Cyprinella caerulea</i>	TUBRI 68572	5	Pelagic	-	HQ557380	GQ275174	-	GQ275313
<i>Cyprinella callisema</i>	TUBRI 26173	5	Pelagic	-	JN025190	GQ275238	-	GQ275301
<i>Cyprinella callistia</i>	TUBRI 18713	5	Pelagic	8	JN025194	HQ446743	-	GQ275296
<i>Cyprinella calitaenia</i>	TUBRI 23324	5	Pelagic	-	-	GQ275242	-	GQ275310
<i>Cyprinella camura</i>	OKMNH 82159	5	Pelagic	-	JN025203	GQ275235	-	GQ275299
<i>Cyprinella forlonensis</i>	TUBRI 5654	5	Pelagic	-	-	-	-	-
<i>Cyprinella galactura</i>	TUBRI 32805	5	Pelagic	-	KF929808	GQ275226	-	GQ275294
<i>Cyprinella garmani</i>	TUBRI 94081	5	Pelagic	-	-	-	-	-
<i>Cyprinella gibbsi</i>	TUBRI 152239	5	Pelagic	-	HQ557419	KF058914	-	GQ275309
<i>Cyprinella labrosa</i>	TUBRI 28252	5	Benthic	9	-	GQ275182	-	GQ275318
<i>Cyprinella leedsi</i>	TUBRI 24745	5	Pelagic	-	HQ557452	GQ275239	-	GQ275302
<i>Cyprinella lepida</i>	OKMNH 64863	5	Pelagic	-	-	GQ275178	-	GQ275273
<i>Cyprinella lutrensis</i>	OKMNH 33156	5	Pelagic	-	EU751769	DQ324095	EU082750	GQ275278
<i>Cyprinella nivea</i>	TUBRI 202661	5	Pelagic	-	JN025235	GQ275241	-	GQ275304

<i>Cyprinella proserpina</i>	OKMNH 72556	5	Pelagic	-	HQ557205	DQ324101	EU082752	EU082601
<i>Cyprinella pyrrhomelas</i>	TUBRI 25931	5	Pelagic	9	JN025246	GQ275246	-	GQ275307
<i>Cyprinella rutila</i>	TUBRI 95962	5	Pelagic	-	EU751774	EU082599	EU082753	EU082599
<i>Cyprinella trichroistia</i>	TUBRI 52160	5	Pelagic	-	HQ579089	HQ446745	-	GQ275305
<i>Cyprinella venusta</i>	OKMNH 48628	5	Pelagic	-	JN025264	AF261218	-	GQ275312
<i>Cyprinella xaenura</i>	TUBRI 117047	5	Pelagic	9	HQ557459	GQ275249	-	GQ275308
<i>Dionda argentosa</i>	OKMNH 72545	5	Benthic	-	-	GU252344	EU082717	JN812574
<i>Dionda ditaboli</i>	TUBRI 43870	5	Benthic	-	-	JN812394	EU082706	JN812595
<i>Dionda episcopa</i>	OKMNH 73282	5	Benthic	-	JN025287	JN812390	EU082715	JN812592
<i>Dionda rascotis</i>	TUBRI 5605	4	Benthic	-	-	-	-	-
<i>Ericymba ampliarmala</i>	TUBRI 203620	5	Benthic	4	HQ557212	KC763688	GU134199	-
<i>Ericymba buccata</i>	TUBRI 174369	5	Benthic	4	KF929860	AY486010	-	GU136300
<i>Erismystax cabri</i>	TUBRI 95652	5	Benthic	-	HQ557200	JQ712318	GU134200	GU136301
<i>Erismystax dissimilis</i>	TUBRI 52425	5	Benthic	-	JN025369	KC763652	-	KC763726
<i>Erismystax harryi</i>	TUBRI 43760	5	Benthic	-	JN025392	AY486042	-	KC763727
<i>Erismystax insignis</i>	TUBRI 69245	5	Benthic	-	JN025409	KC763653	GU134205	GU136306
<i>Erismystax x-punctatus</i>	OKMNH 61687	5	Benthic	-	JN025432	JX442989	JX443218	JX443320
<i>Exoglossum laurae</i>	TUBRI 76153	5	Benthic	-	HQ557360	KC763683	JX443217	JX443319
<i>Exoglossum maxillingua</i>	TUBRI 575	4	Benthic	-	EU524616	-	-	-
<i>Falcularius chapulae</i>	TUBRI 30790	5	Pelagic	-	-	JX443024	JX443251	JX443354
<i>Gila atraria</i>	TUBRI 8899	2	Pelagic	-	-	KF514155	JX443255	JX443358
<i>Gila coerulea</i>	TUBRI 121794	5	Pelagic	-	JN026719	KF514185	JX443256	JX443359
<i>Gila cypha</i>	TUBRI 95166	3	Pelagic	-	-	-	JX443248	JX443352
<i>Gila ditaeana</i>	OKMNH 41962	5	Pelagic	-	-	KF514209	JX443265	JX443368
<i>Gila modesta</i>	TUBRI 28741	5	Pelagic	-	-	KF514226	KF514329	GU136311
<i>Gila nigrescens</i>	TUBRI 54481	5	Pelagic	-	HQ557461	EU747198	KF514330	JX443367
<i>Gila pandora</i>	TUBRI 104123	5	Pelagic	-	JN026723	JX443021	JX443247	JX443350
<i>Gila purpurea</i>	TUBRI 124541	5	Pelagic	-	-	KF514255	JX443258	JX443350
<i>Gila robusta</i>	TUBRI 95801	4	Pelagic	-	JN026724	KF514255	JX443258	JX443363
<i>Gila seminata</i>	TUBRI 96661	1	Pelagic	-	-	KF514256	JX443241	JX443346
<i>Hemitremia flammea</i>	TUBRI 129162	5	Pelagic	-	JN026753	AY281054	GU134215	GU136316
<i>Hesperoleucus symmetricus</i>	TUBRI 17244	5	Pelagic	1	JN026764	JX443087	JX443309	JX443412
<i>Hybognathus hankinsoni</i>	OKMNH 72252	5	Benthic	7	EU524081	EU811090	EU811144	KC763703
<i>Hybognathus hayi</i>	TUBRI 63773	5	Benthic	7	-	EU811092	EU811146	-
<i>Hybognathus nuchalis</i>	OKMNH 73671	5	Benthic	7	HQ557721	EU811096	GU134217	EU711120
<i>Hypopsis albus</i>	TUBRI 30666	5	Benthic	-	-	-	-	-

<i>Hybopsis_amblops</i>	OKMNH 81416	5	Benthic	5	KF929979	HQ446747	GU134214	GU136315
<i>Hybopsis_ammis</i>	OKMNH 77509	5	Benthic	5	KF929980	EU917442	-	KC76372
<i>Hybopsis_dorsalis</i>	OKMNH 64405	5	Benthic	5	JN027534	KC763661	-	KC763755
<i>Hybopsis_lineapunctata</i>	TUBRI 168131	5	Benthic	5	KF929981	EU917428	-	KC763724
<i>Hybopsis_rubrifrons</i>	TUBRI 116946	5	Benthic	5	KF929982	EU917433	-	-
<i>Hybopsis_wincbelli</i>	TUBRI 55035	5	Benthic	5	KF929983	GQ275173	JX443212	KC763725
<i>Lavinia_exitlicauda</i>	TUBRI 42874	5	Pelagic	1	KF558317	JX443003	GU134221	GU136320
<i>Lepidomeda_copei</i>	TUBRI 104176	5	Pelagic	-	-	KJ175027	-	AY825461
<i>Lepidomeda_mollispinis</i>	TUBRI 93876	5	Pelagic	-	-	AF452092	-	-
<i>Lepidomeda_vittata</i>	TUBRI 97960	5	Pelagic	-	-	JX443056	JX443284	JX443388
<i>Luxilus_albeolus</i>	OKMNH 77483	5	Pelagic	5	JN027087	-	-	KC763376
<i>Luxilus_cardinalis</i>	OKMNH 79670	5	Pelagic	5	-	U66601	-	KC763744
<i>Luxilus_cerasinus</i>	OKMNH 77487	5	Pelagic	5	HQ557208	U66599	GU134219	GU136319
<i>Luxilus_chrysocephalus</i>	OKMNH 70900	5	Pelagic	5	EU524764	GQ275161	GU134220	EF452829
<i>Luxilus_coccogenis</i>	TUBRI 72115	5	Pelagic	5	JN027100	U66603	-	KC763770
<i>Luxilus_cornutus</i>	OKMNH 45500	5	Pelagic	5	HQ557724	U66597	-	KC763747
<i>Luxilus_pilsbryi</i>	OKMNH 43252	5	Pelagic	5	-	U66602	-	KC763745
<i>Luxilus_zonatus</i>	OKMNH 43370	5	Pelagic	5	JN027112	U66600	-	KC763746
<i>Luxilus_zonistius</i>	TUBRI 174587	5	Pelagic	5	JN027113	U66604	-	KC763772
<i>Lythrurus_ardens</i>	OKMNH 77387	5	Pelagic	5	KF930089	U17268	-	-
<i>Lythrurus_bellus</i>	TUBRI 35301	5	Pelagic	5	JN027125	U17275	-	KC763741
<i>Lythrurus_fasciolaris</i>	TUBRI 197843	5	Pelagic	5	HQ557213	HQ446749	-	KC763739
<i>Lythrurus_fumeus</i>	OKMNH 78335	5	Pelagic	5	KF930092	U17269	GU134222	GU136321
<i>Lythrurus_lirus</i>	TUBRI 59871	5	Pelagic	5	JN027145	U17273	-	KC763740
<i>Lythrurus_roseipinnis</i>	TUBRI 144804	5	Pelagic	5	KF930093	X66456	-	KC763742
<i>Lythrurus_snelsoni</i>	OKMNH 70884	5	Pelagic	5	JN027163	U17272	-	-
<i>Lythrurus_umbraealis</i>	OKMNH 78177	5	Pelagic	5	EU524793	GQ275160	GU134223	GU136322
<i>Macrhybopsis_aestivaalis</i>	TUBRI 12066	5	Benthic	5	HQ579090	JQ712319	GU134225	GU136325
<i>Macrhybopsis_australis</i>	OKMNH 43756	5	Benthic	5	-	-	-	-
<i>Macrhybopsis_gelida</i>	TUBRI 42837	1	Benthic	-	JN027179	-	-	-
<i>Macrhybopsis_hyostoma</i>	OKMNH 75222	6	Benthic	-	JN027183	-	-	-
<i>Macrhybopsis_marcconis</i>	OKMNH 64816	5	Benthic	-	JN027185	-	-	-
<i>Macrhybopsis_meeki</i>	TUBRI 192101	3	Benthic	-	KF930095	-	-	-
<i>Macrhybopsis_storeriana</i>	OKMNH 69618	10	Benthic	-	EU524801	-	GU134230	GU136329
<i>Margariscus_margarita</i>	TUBRI 197999	5	Benthic	5	HQ557395	JX443011	JX443236	JX443339
<i>Margariscus_nachtriebii</i>	TUBRI 5307	4	Benthic	-	-	-	-	-

<i>Meda fulgida</i>	TUBRI 90765	5	Benthic	-	HQ556952	JX443055	JX443279	JX443382
<i>Mocpa coriacea</i>	TUBRI 47262	5	Pelagic	-	HQ557334	JX443064	JX443287	JX443390
<i>Mylopharodon conocephalus</i>	TUBRI 70301	5	Pelagic	1	-	JX443005	JX443231	JX443333
<i>Nocomis effusus</i>	TUBRI 89474	5	Benthic	-	JN027337	KM281541	KM281607	KC763733
<i>Nocomis micropogon</i>	TUBRI 191507	5	Benthic	-	JN027359	JQ712348	KM281617	KC763734
<i>Nocomis platyrhynchus</i>	TUBRI 10504	5	Benthic	-	HQ579092	KC763685	-	KC763735
<i>Nocomis raneyi</i>	TUBRI 72636	5	Benthic	-	JN027367	JQ712349	KM281618	GQ275254
<i>Notropis alborus</i>	TUBRI 75467	5	Pelagic	-	-	KC763686	-	KC763712
<i>Notropis altipinnis</i>	TUBRI 71870	5	Pelagic	-	JN027375	KC763655	-	KC763748
<i>Notropis annophilus</i>	TUBRI 83597	5	Benthic	4	HQ579093	AF117161	-	KC763717
<i>Notropis amoenus</i>	TUBRI 25851	5	Pelagic	-	JN027395	AF352270	-	KC763750
<i>Notropis anogenus</i>	TUBRI 69849	3	Pelagic	4	EU524949	AY140698	-	KC763716
<i>Notropis aquirepequilloi</i>	TUBRI 84800	5	Pelagic	-	-	-	-	-
<i>Notropis arionmus</i>	OKMNH 36561	5	Pelagic	-	HQ557207	AY281057	-	KC763762
<i>Notropis asperifrons</i>	TUBRI 32744	5	Pelagic	7	HQ557182	HQ446751	GU134231	GU136330
<i>Notropis atherinoides</i>	OKMNH 60901	5	Pelagic	-	KF930185	HM224297	GU134232	EF452832
<i>Notropis atrocaudalis</i>	OKMNH 74847	5	Pelagic	-	JN027431	KC763687	-	KC763702
<i>Notropis baileyi</i>	TUBRI 76935	5	Pelagic	7	JN027442	KC763656	-	EU292691
<i>Notropis bairdi</i>	OKMNH 79756	5	Pelagic	7	JN027447	KC763657	-	-
<i>Notropis bifrenatus</i>	TUBRI 23217	5	Pelagic	4	KF930186	KC763658	-	-
<i>Notropis blennioides</i>	OKMNH 67484	5	Pelagic	7	JN027453	AF117171	GU134234	GU136331
<i>Notropis boops</i>	OKMNH 71192	5	Pelagic	-	KF930187	AF352261	-	KC763714
<i>Notropis braytoni</i>	OKMNH 31279	5	Pelagic	7	-	GQ275168	-	GQ275264
<i>Notropis buchananii</i>	OKMNH 62030	5	Pelagic	-	EU524980	HM179629	-	GQ275269
<i>Notropis cahabae</i>	TUBRI 69102	5	Pelagic	-	HQ557188	KC763659	-	KC763709
<i>Notropis calientis</i>	TUBRI 31909	5	Pelagic	-	-	GQ249853	EU082735	EU082583
<i>Notropis candidus</i>	TUBRI 130763	5	Pelagic	7	-	AF352275	-	-
<i>Notropis chalybaeus</i>	TUBRI 1809	5	Pelagic	7	JN027492	KC763697	-	KC763704
<i>Notropis chihuahua</i>	TUBRI 95917	5	Pelagic	-	HQ579028	KC763698	-	KC763710
<i>Notropis chiliticus</i>	TUBRI 29832	5	Pelagic	7	JN027505	KC763689	-	KC763769
<i>Notropis chlorocephalus</i>	TUBRI 199572	5	Pelagic	7	JN027508	KC763690	-	KC763765
<i>Notropis chrosomus</i>	TUBRI 176137	5	Pelagic	7	JN027517	AF352262	-	KC763767
<i>Notropis cummingsae</i>	TUBRI 97895	5	Pelagic	-	HQ557185	KC763660	-	KC763757
<i>Notropis edwardsianeyi</i>	TUBRI 166024	5	Pelagic	7	JN027535	AF352263	-	-
<i>Notropis girardi</i>	OKMNH 81344	5	Pelagic	-	JN027541	AF352276	-	-
<i>Notropis greeni</i>	OKMNH 43235	5	Pelagic	-	JN027542	KC763662	-	KC763721

<i>Notropis harperi</i>	TUBRI 38202	5	Benthic	6	JN027546	KC763663	GU134235	GU136332
<i>Notropis heterodon</i>	TUBRI 14453	5	Pelagic	-	KF930188	AY140697	-	KC763715
<i>Notropis heterolepis</i>	TUBRI 11393	5	Pelagic	-	EU524999	AY140696	-	KC763700
<i>Notropis hudsonius</i>	TUBRI 20258	5	Pelagic	3	KF930189	HQ446752	-	-
<i>Notropis hypsilepis</i>	TUBRI 181288	5	Pelagic	-	JN027563	-	-	-
<i>Notropis jemezianus</i>	OKMNH 65168	5	Pelagic	-	JN027565	AF352277	-	-
<i>Notropis leuciodus</i>	TUBRI 203949	5	Pelagic	-	JN027566	HQ446753	GU134237	GU136334
<i>Notropis longirostris</i>	TUBRI 150035	5	Benthic	-	KF930190	AF352264	GQ184535	KC763718
<i>Notropis lutipinnis</i>	TUBRI 168531	5	Pelagic	7	JN027592	KC763664	-	KC763766
<i>Notropis maculatus</i>	OKMNH 29715	5	Pelagic	-	JN027600	KC763691	GU134238	GU136335
<i>Notropis matutinus</i>	TUBRI 34455	5	Pelagic	-	-	-	-	-
<i>Notropis melis-tocholas</i>	TUBRI 38549	4	Benthic	-	JN027602	KC763665	-	-
<i>Notropis melanostomus</i>	TUBRI 98579	5	Pelagic	5	-	KC763666	-	-
<i>Notropis nazas</i>	TUBRI 202471	5	Benthic	7	JN027605	KC763667	GU134239	GU136336
<i>Notropis nubius</i>	OKMNH 75301	5	Benthic	-	KF930191	HM245602	-	GQ275270
<i>Notropis orientburgeri</i>	OKMNH 80838	6	Pelagic	-	JN027614	KC763692	GU134240	GU136337
<i>Notropis percobromus</i>	OKMNH 82381	5	Pelagic	-	EU524177	EU084780	-	KC763752
<i>Notropis petersoni</i>	TUBRI 24634	5	Pelagic	-	JN027624	KC763669	-	KC763773
<i>Notropis photogenis</i>	TUBRI 19432	5	Pelagic	-	EU525015	AF352281	-	KC763743
<i>Notropis potteri</i>	OKMNH 61107	5	Pelagic	7	JN027634	AF352266	-	-
<i>Notropis rafinesquei</i>	TUBRI 87671	5	Benthic	4	-	AF117187	-	KC763774
<i>Notropis rubellus</i>	OKMNH 71848	5	Pelagic	-	HQ557389	EU084867	-	-
<i>Notropis rubricroceus</i>	TUBRI 29602	5	Pelagic	7	JN027653	KC763671	-	KC763768
<i>Notropis sabinae</i>	TUBRI 63283	4	Benthic	-	JN027662	AF117199	-	KC763719
<i>Notropis scabriceps</i>	TUBRI 70091	5	Pelagic	-	HQ557321	KC763672	-	KC763722
<i>Notropis scepiticus</i>	TUBRI 29822	4	Pelagic	-	JN027672	AF352283	-	KC763754
<i>Notropis semperasper</i>	TUBRI 72847	5	Pelagic	-	JN027677	KC763673	-	KC763764
<i>Notropis shumardi</i>	OKMNH 29896	5	Pelagic	7	JN027681	AF352284	-	-
<i>Notropis spectranculus</i>	TUBRI 38294	5	Pelagic	-	HQ579082	KC763674	-	KC763707
<i>Notropis stibius</i>	TUBRI 176302	5	Pelagic	-	JN027702	AF352286	GU134241	GU136338
<i>Notropis stramineus</i>	OKMNH 81946	10	Pelagic	-	KF930193	HM179637	-	KC763711
<i>Notropis suttkusi</i>	OKMNH 81367	5	Pelagic	-	AY116186	EU084873	-	-
<i>Notropis telescopus</i>	TUBRI 169136	5	Pelagic	-	JN027718	AF352290	-	KC763763
<i>Notropis topeta</i>	TUBRI 54082	5	Pelagic	-	JN027732	KC763675	-	-
<i>Notropis tropicus</i>	TUBRI 84843	5	Benthic	8	JQ935926	-	-	-
<i>Notropis uranoscopus</i>	TUBRI 69095	5	Pelagic	-	JN027740	KC763693	-	KC763713

<i>Notropis volucellus</i>	OKMNH 81398	5	Pelagic	-	EU525038	HM179596	GU134242	GU136340
<i>Notropis xaenoecephalus</i>	TUBRI 57831	5	Pelagic	7	HQ557190	HQ446757	-	KC763705
<i>Opsopoeodus emiliae</i>	TUBRI 114644	5	Pelagic	-	JN027856	GQ184498	GU134246	GU136342
<i>Oregonichthys crameri</i>	TUBRI 121769	5	Benthic	-	HQ557331	KC763677	GU134244	GU136341
<i>Orthodon microlepidotus</i>	TUBRI 17243	2	Pelagic	-	-	JX443006	GU134248	GU136344
<i>Phenacobius catostomus</i>	TUBRI 29136	5	Benthic	-	-	HQ446758	GU134249	GU136345
<i>Phenacobius mirabilis</i>	OKMNH 77067	5	Benthic	-	HQ557376	JF949845	GU134257	GU136352
<i>Phenacobius teretulus</i>	TUBRI 70475	5	Benthic	-	HQ579110	JF949844	-	KC763730
<i>Phenacobius uranops</i>	TUBRI 69258	5	Benthic	-	JN028207	JF949843	-	KC763729
<i>Phoxinus eos</i>	TUBRI 197998	5	Pelagic	-	JN028211	EU755056	-	-
<i>Phoxinus oreas</i>	OKMNH 77627	5	Pelagic	-	JN028223	EU755048	-	-
<i>Pimphales notatus</i>	OKMNH 82091	5	Pelagic	-	KF930262	GQ184518	GQ184556	GU136353
<i>Pimphales promelas</i>	OKMNH 72708	5	Pelagic	-	JX516786	GQ184521	AY325788	AY430210
<i>Pimphales tenellus</i>	OKMNH 73015	5	Pelagic	-	JN028242	GQ184527	GQ184551	KC763699
<i>Pimphales vigilax</i>	OKMNH 48068	5	Pelagic	-	GQ557497	GQ184534	GQ184542	GU136356
<i>Plagopterus argentissimus</i>	TUBRI 95807	5	Benthic	-	HQ556962	JX442991	JX443220	JX443322
<i>Platybio gracilis</i>	OKMNH 64457	5	Benthic	-	JN028255	JX442992	GU134252	GU136348
<i>Pteronotropis eurzonus</i>	TUBRI 175373	5	Pelagic	6	HQ557317	AF261223	GU134251	KJ634259
<i>Pteronotropis grandipinnis</i>	TUBRI 189315	5	Pelagic	6	HQ579010	KC763695	KM048266	KJ634248
<i>Pteronotropis hubbsi</i>	TUBRI 18801	5	Pelagic	6	JN028299	KC763679	GU134254	KJ634255
<i>Pteronotropis hypselopterus</i>	TUBRI 151593	5	Pelagic	6	HQ971430	HM224303	KM048258	KJ634240
<i>Pteronotropis merlini</i>	TUBRI 189458	5	Pelagic	6	-	KC763696	KM048268	KC763760
<i>Pteronotropis metallicus</i>	TUBRI 197908	5	Pelagic	6	HQ937029	KC763694	KM048279	KJ634261
<i>Pteronotropis welaka</i>	TUBRI 66194	5	Pelagic	6	HQ579008	KC763681	KM048275	KJ634257
<i>Ptychocheilus grandis</i>	TUBRI 42851	5	Benthic	1	KF558277	JX443070	JX443188	JX443396
<i>Ptychocheilus oregonensis</i>	TUBRI 54251	5	Benthic	1	JQ354306	JX443068	JX443291	JX443394
<i>Ptychocheilus umpqua</i>	TUBRI 121770	5	Benthic	1	JN028328	JX443066	JX443289	JX443392
<i>Relictus solitarius</i>	TUBRI 94344	5	Pelagic	1	-	JN997101	JN997369	GU136360
<i>Rhinichthys atratulus</i>	OKMNH 77792	5	Benthic	-	JX517178	KF640095	KF640179	GU136357
<i>Rhinichthys cataractae</i>	TUBRI 38019	5	Benthic	-	KF930359	KF640157	KF640208	JX443315
<i>Rhinichthys evermanni</i>	TUBRI 43677	4	Benthic	-	JN028367	EU780890	-	-
<i>Rhinichthys obtusus</i>	TUBRI 3865	5	Benthic	-	JX517192	DQ990250	-	KC763732
<i>Rhinichthys occulus</i>	TUBRI 93828	5	Benthic	-	-	-	-	-
<i>Richardsonius balteatus</i>	TUBRI 99141	5	Benthic	2	JN028395	KJ68429	GU182705	EF452835
<i>Semotilus corporalis</i>	TUBRI 36783	5	Benthic	-	EU525152	-	-	-
<i>Semotilus thoreauianus</i>	TUBRI 199037	5	Benthic	-	HQ557175	-	GU134269	GU136363

<i>Siphateles bicolor</i>	TUBRI 121802	5	Pelagic	1	-	AF237751	JX443305	JX443408
<i>Tampichthys erimyzonops</i>	TUBRI 38974	4	Benthic	8	JQ935935	DQ324071	EU082687	EU082536
<i>Tampichthys ipni</i>	TUBRI 84818	5	Benthic	8	JQ935940	EU082480	GU134197	GU136298

FIGURE S1

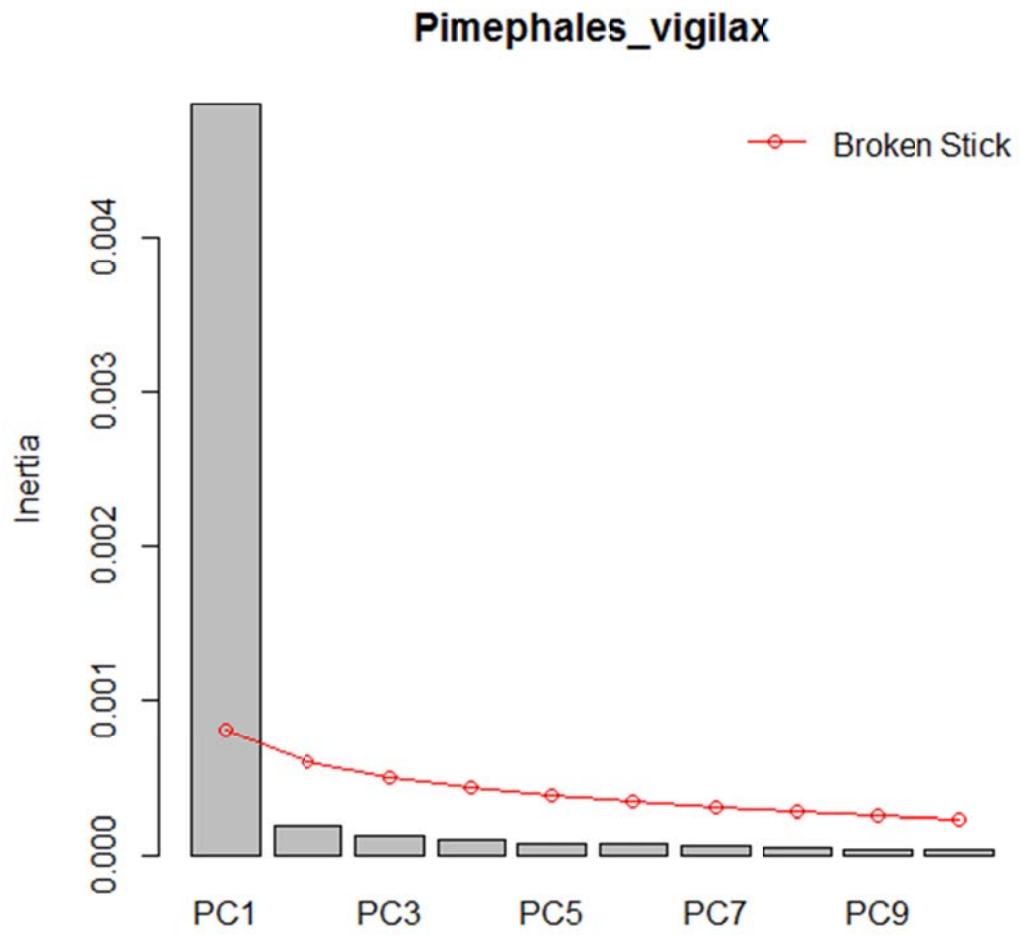


FIGURE S2

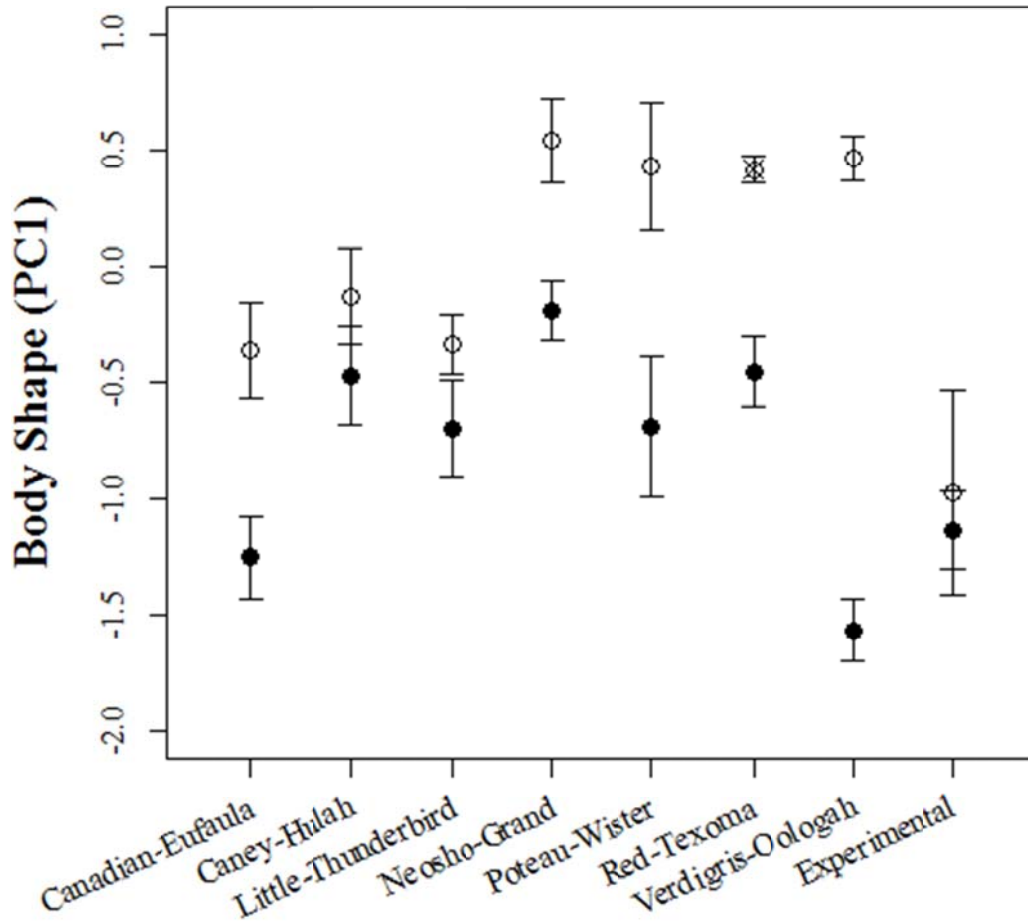


FIGURE S3



FIGURE S4

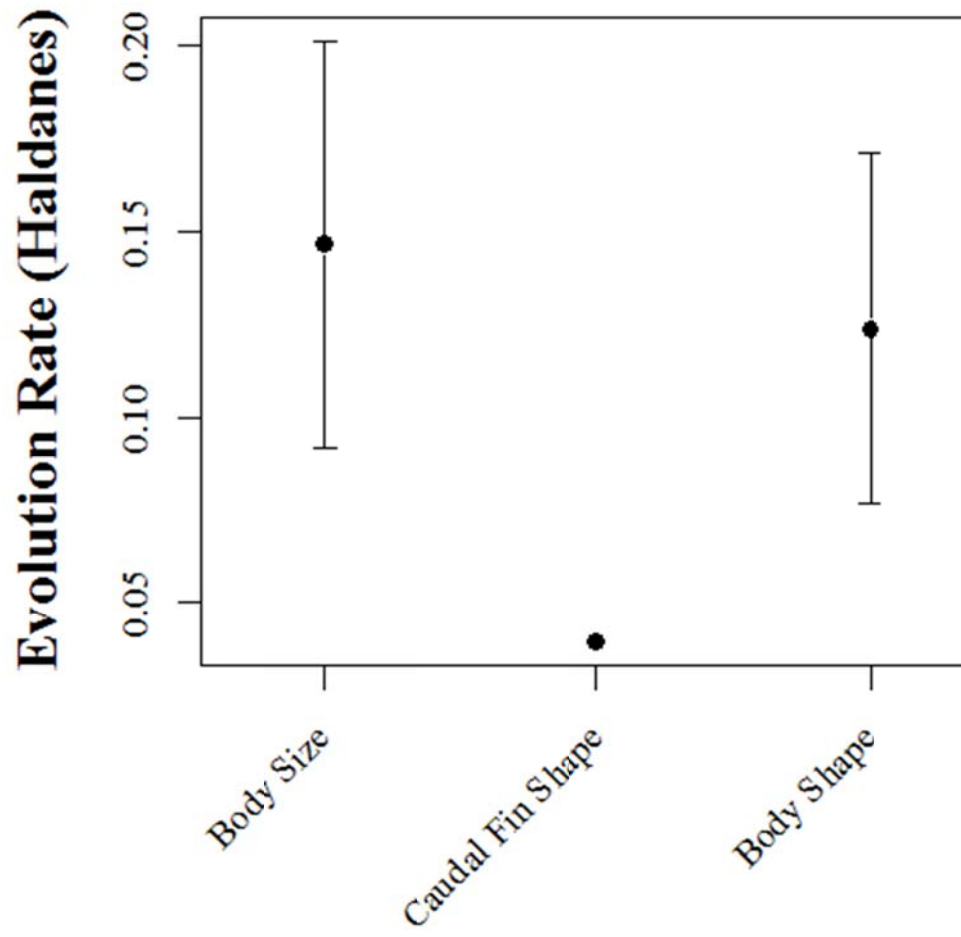


FIGURE S5

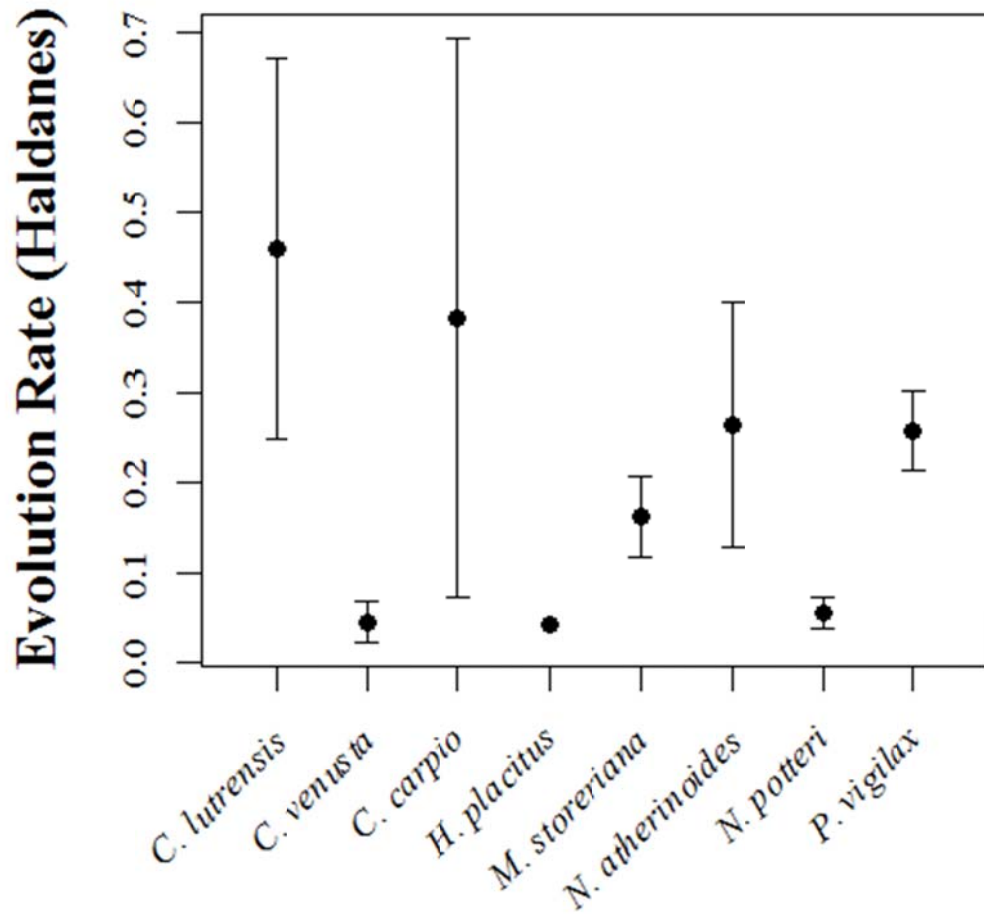


Figure S6

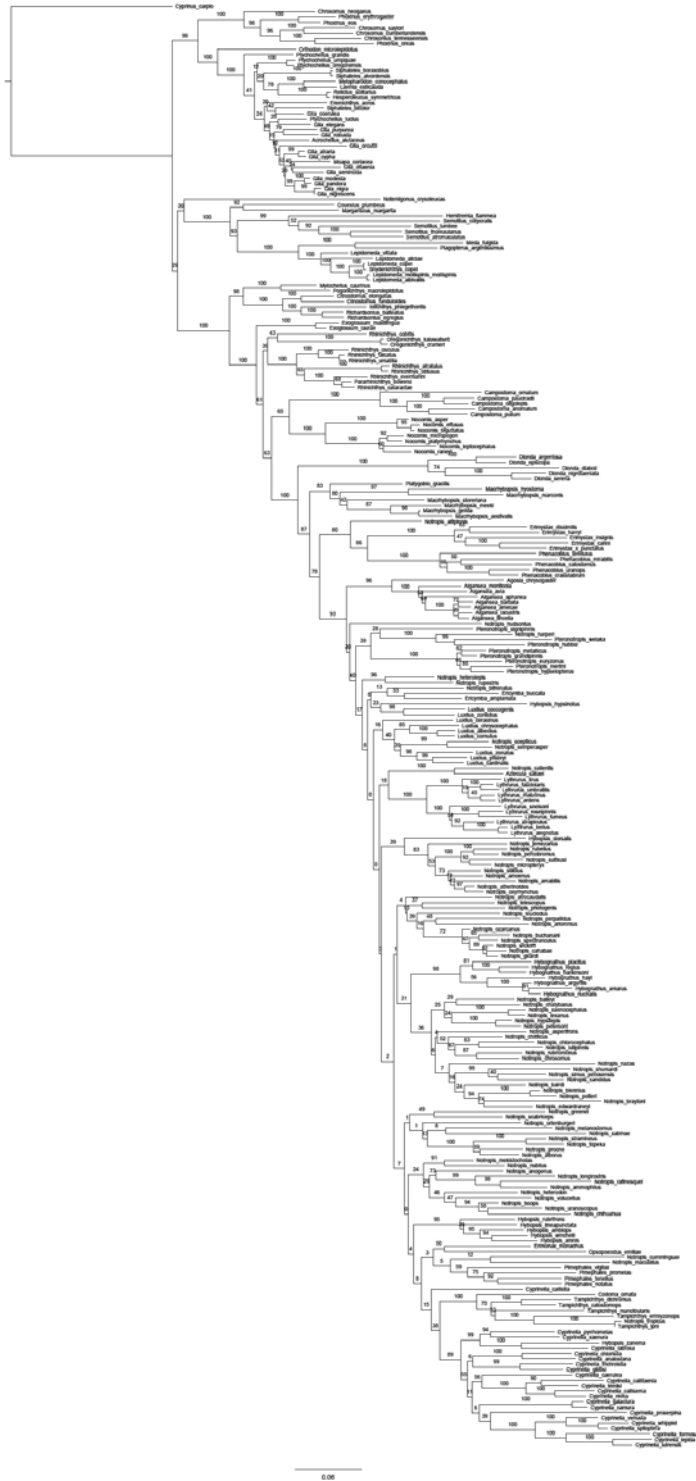


FIGURE S7

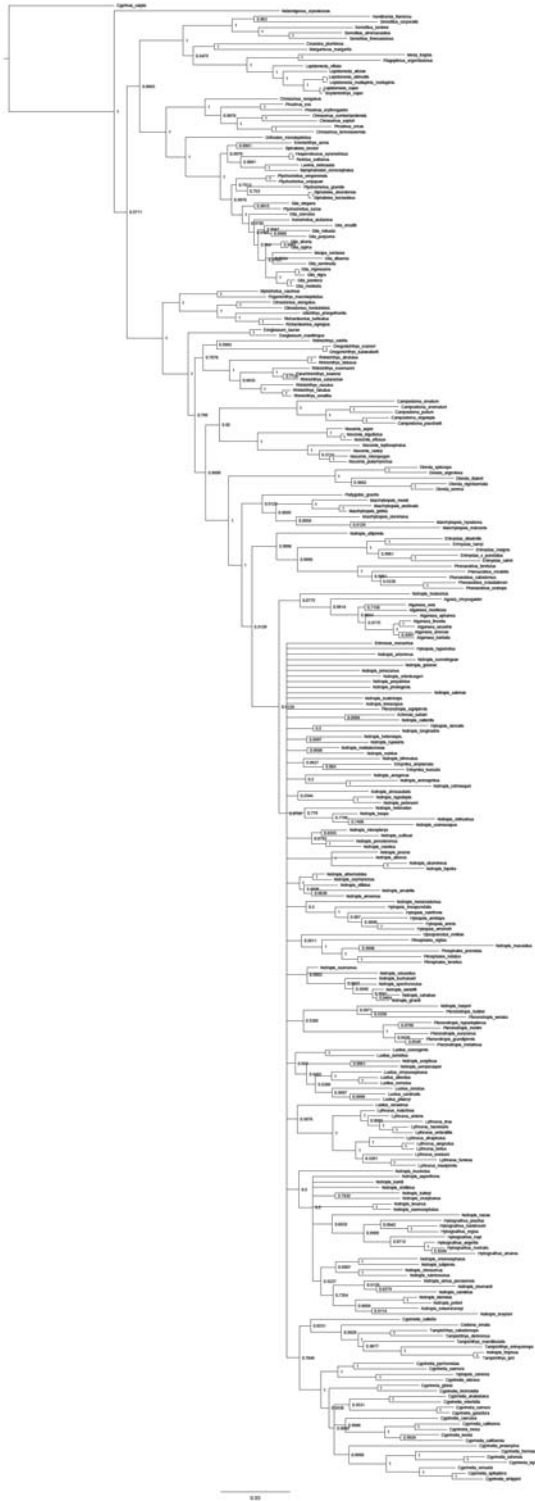


FIGURE S8

