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Shaping up: a geometric morphometric approach to assemblage ecomorphology

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This study adopts an ecomorphological approach to test the utility of body shape as a predictor of niche relationships among a stream fish assemblage of the Tickfaw River (Lake Pontchartrain Basin) in southeastern Louisiana, U.S.A. To examine the potential influence of evolutionary constraints, analyses were performed with and without the influence of phylogeny. Fish assemblages were sampled throughout the year, and ecological data (habitat and tropic guild) and body shape (geometric morphometric) data were collected for each fish specimen. Multivariate analyses were performed to examine relationships and differences between body shape and ecological data. Results indicate that a relationship exists between body shape and trophic guild as well as flow regime, but no significant correlation between body shape and substratum was found. Body shape was a reliable indicator of position within assemblage niche space.

Key words: body shape; community ecology; ecomorphology; fish; habitat; trophic guild.

INTRODUCTION

Ecologists have long been intrigued by the ecological and historical processes that drive ecomorphological patterns and assemblage structure. To address the mechanisms behind community assemblage structure, researchers have often taken an ecomorphological approach, which is the idea that the morphology is related to, or indicative of, the ecological role or niche of an organism (Ricklefs & Miles, 1994). Ecomorphology assumes species that occupy similar ecological niches share key morphological attributes due to adaptation to similar selective pressures, thereby allowing inferences about the ecology of species to be made from morphological characteristics (Gatz, 1979*a*; Douglas, 1987; Motta & Kotrschal, 1992; Hugueny & Pouilly, 1999; Oliveira *et al.*, 2010). While morphology can act as a surrogate for the fundamental ecological niche, the realized ecological niche is determined by various biotic and abiotic factors, which can restrict the realized niche of an organism (Hutchinson, 1957). The use of morphological traits as a surrogate for niche position and the ecomorphological patterns given by these traits has been studied in a variety of taxa (Gatz, 1979*b*; Swartz *et al.*, 2003; França *et al.*, 2008; Inward *et al.*, 2011; Baraloto *et al.*, 2012).

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Several researchers have suggested that some patterns of ecomorphology may be an artefact of the phylogenetic relationships rather than environmental processes (Douglas & Matthews, 1992; França *et al.*, 2008). Therefore, exploring the influence of phylogeny is an integral component of ecomorphological studies because morphological similarity may be due to shared ancestry rather than selective pressures (Winemiller, 1991; Losos & Miles, 1994; Guill *et al.*, 2003).

Fishes provide an excellent model group in which to study the intersection between morphology and ecology. Gatz (1979*b*) was among the first to formally examine various functional morphological traits of fish assemblages and the covariance of these traits with the ecologies of fishes. Many studies have since shown a robust relationship between morphological traits and various trophic ecologies, swimming ability and habitat use of fishes (Gatz, 1979*b*; Douglas, 1987; Winemiller, 1991; Douglas & Matthews, 1992; Wagner *et al.*, 2009; Cochran-Biederman & Winemiller, 2010). Specifically, some studies have shown strong relationships between diet and morphological traits such as digestive tract length, gill raker size and body shape (Gatz, 1979*b*; Cochran-Biederman & Winemiller, 2010). Although some authors have found weak or indeterminate relationships between morphology and habitat use in fishes (Oliveira *et al.*, 2010), there are many widely established relationships. Morphological features have been widely used to determine habitat and trophic dimensions of a niche (Gatz, 1981; Ingram & Shurin, 2009; Maldonado *et al.*, 2009; Oliveira *et al.*, 2010). MacArthur (1968) suggested a parallel between niche and phenotypes of a species. This suggests the ability to predict the position of a species in an assemblage niche space using morphological traits.

The idea that niche utilization is correlated or constrained by phenotype is an underlying assumption of ecomorphology. This assumption would lead to expect that similar ecomorphotypes exploit similar habitat types and trophic guilds. An ecomorphotype is defined as a group of individuals that exploit similar resources using similar morphological traits, independent of their phylogenetic relationships (Oliveira *et al.*, 2010). Although there is evidence for correlations between morphology and ecology, no generalized ecomorphotype models have been created to predict the habitat choices of temperate fish assemblages (Oliveira *et al.*, 2010).

Ecomorphology assumes that species occupying similar niches would have similar morphologies due to adaptation to similar selective pressures. Douglas & Matthews (1992) defended the idea that ecomorphological studies of North American stream fishes should be restricted to within the family level due to the limiting effects of evolutionary history in the detection of morphological adaptations; finding that morphological similarity was more due to taxonomic relatedness than convergence of morphological traits *via* adaptation to selective pressures. Hugueny & Pouilly (1999) and Oliveira *et al.* (2010), however, refuted this idea, finding that, in spite of historical influence, ecomorphological studies are a valid evaluation of assemblage structure for tropical fishes.

Morphometrics is a quantitative way of comparing the shape of two or more objects or organisms. Traditional morphometrics use linear measurements of depth, width and length for a quantitative analysis, but this provides relatively little information about the actual shape of an organism (Rohlf & Marcus, 1993). In addition, traditional morphometrics do not accurately separate shape from size-based differences (Zelditch *et al.*, 2004). Geometric morphometrics is an alternative approach that is more effective at capturing meaningful information about the shape of an organism and uses statistical analyses that are more powerful and make fewer assumptions relative to importance of measurements (Foster *et al.*, 2008; Cooper & Westneat, 2009). Geometric morphometric analyses have been widely applied in evolutionary and ecological studies. For example, Cooper & Westneat (2009) used geometric morphometrics to study trophic niches in damselfishes (Pomacentridae), Foster *et al.* (2008) analysed beak shape in Darwin's finches and Kassam *et al.* (2003) and Russo *et al.* (2008) studied resource partitioning of fishes. Studies have shown a link between the body shape and resource use of fish species using geometric morphometrics (Kassam *et al.*, 2003; Maldonado *et al.*, 2009), but there is a lack of studies examining the covariance of body shape with diet and habitat use for a complete assemblage using geometric morphometrics and phylogenetics. Body shape and size are two of the most important factors in structuring the ecological niche of stream fishes and the functioning of an assemblage (Douglas, 1987). Body shape can be used as a surrogate for the niche position of fish species, yet there is a discrepancy regarding whether body shape is a valid test of niche position across multiple families in an assemblage of fishes (Douglas & Matthews, 1992; Oliveira *et al.*, 2010).

This study examines ecomorphological patterns of species of fishes in a Gulf Of Mexico coastal-plain river in south-eastern Louisiana, U.S.A. Specifically, the study focuses on two questions. First, is there a correlation between body shape and the trophic guild using geometric morphometrics of the fishes in a stream assemblage with and without the influence of phylogenetic relationships? Second, using geometric morphometrics, is there a correlation between body shape and the habitat of the species in this assemblage with and without the influence of phylogenetic relationships?

MATERIALS AND METHODS

SAMPLING AND HABITAT QUANTIFICATION

Fishes were collected in the Tickfaw River (Lake Pontchartrain Basin) in south-eastern Louisiana (30° 50′ 446″ N; 90° 55′ 417″ W) from 2011 to 2012 in the months of November, April and July using a backpack electrofisher. In an attempt to sample most of the heterogeneous habitats in the site, the study site was sectioned into 13 transects located one mean stream width apart, where each transect was sampled once per sample period (Simonson *et al.*, 1994). Backpack electrofishing runs across transects were conducted to sample fishes. At the initial encounter of a fish in each transect, a 1 m radius, roughly estimated using a backpack electrofishing pole, was sampled, and all fishes collected were considered to be from this sample point. Each sample point was marked with a flag. Any fishes collected outside of this sample point were counted as another sample point. This sampling method was continued for all transects. All specimens were anaesthetized using MS-222 and preserved in 10% formalin. At each marked sample point, ecological data were taken: flow (cm s^{-1}), temperature (° C), dissolved oxygen (mg l^{-1}), water depth (cm) and the dominate substratum type broadly identified by presences in sample point: sand, debris, gravel, sand-debris, sand-gravel and silt.

GEOMETRIC MORPHOMETRIC ANALYSES

Lateral photographs were taken of each specimen with a Nikon D5000 SLR camera (www.nikon.com). Standard length (L_S) of each specimen was also measured with callipers to the nearest 0·01 mm. Juvenile and bent specimens were removed from analysis to limit possible ontogenetic effects and preservation of artefacts. tpsDig 2.10 (Rohlf, 2006) software was used to generate the co-ordinate landmarks. Twelve homologous landmarks and two supplementary

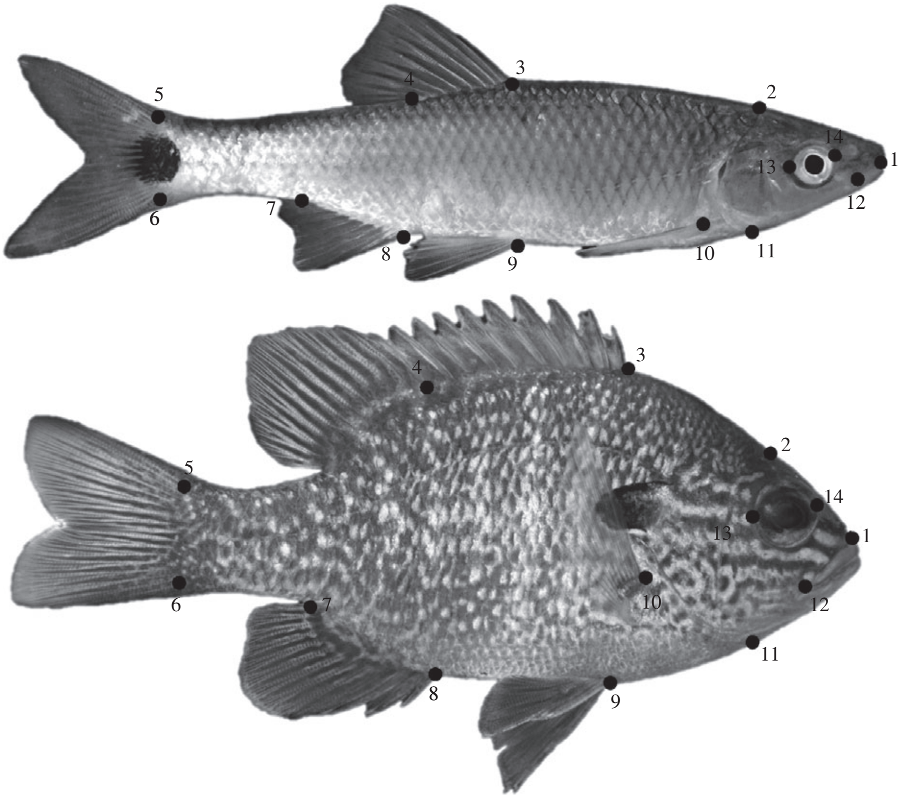

Fig. 1. Eleven homologous landmarks and three supplementary measurements gathered to capture overall body shape: 1, anterior tip of the snout; 2, posterior aspect of the neurocranium; 3, anterior origin of the spinous dorsal fin; 4, posterior insertion of the dorsal fin or spiny-fin dorsal fin; 5, dorsal insertion of the caudal fin; 6, ventral insertion of the caudal fin; 7, posterior insertion of the anal fin; 8, anterior insertion of the anal fin; 9, origin of the pelvic fin; 10, upper insertion of the pectoral fin; 11 and 12, outer edges of eye parallel to a line drawn between points 1 and 10; 13, posterior corner of maxilla; 14, the insertion of the operculum on the profile.

measurements were gathered to capture overall body shape (Rohlf, 2006). All landmarks were digitized on the right side of the fishes (Fig. 1). Voucher specimens were deposited in the Southeastern Louisiana University Vertebrate Museum (SLU).

Body shape

Biases introduced by position, orientation and size were removed by using a Procrustes superimposition method in MorphoJ 1.05d (Klingenberg, 2011). The Procrustes method superimposes configurations of landmarks of all specimens to achieve the best overall fit (Klingenberg & McIntyre, 1998). Principal component analyses (PCA) were performed on each family in MorphoJ to reduce the dimensionality of the data and to identify outliers (any specimens not belonging to 95% confidence ellipses) following Clabaut *et al.* (2007). Twenty-three outliers from 12 species were removed before any further analyses.

Trophic guilds

To establish trophic guild membership, dietary information and life-history information was obtained from the literature for all species analysed (Table I). Using this information, six general

TABLE I. The number of individuals, trophic guilds, substratum modes and source of information for all species

Family	Species	Number	Diet	Substratum	Source
Cyprinidae	*Cyprinella venusta*	109	Generalist omnivores	Sand	Hambrick & Hibbs (1977)
	Hybopsis winchelli	22	Benthic omnivores	Sand debris	Davis & Miller (1967)
	Luxilus chrysocephalus	1	N/A	N/A	N/A
	Notropis longirostris	44	Benthic omnivores	Sand	Heins & Clemmer (1975)
	Notropis texanus	7	Generalist omnivores	Sand	Becker (1983), Felley & Felley (1987)
	Opsopoeodus emiliae	2	N/A	N/A	N/A
	Pimephales vigilax	8	Benthic omnivores	Sand debris	Parker (1964), Whitaker (1977)
Ictaluridae	*Ictalurus punctatus*	2	Benthic omnivores	N/A	N/A
	Noturus leptacanthus	10	Benthic invertivores	Sand debris	Chan & Parsons (2000)
	Noturus miurus	1	N/A	N/A	N/A
Catostomidae	*Hypentelium nigricans*	3	N/A	N/A	N/A
	Moxostoma poecilurum	3	N/A	N/A	N/A
Atherinopsidae	*Labidesthes sicculus*	4	N/A	N/A	N/A
Poeciliidae	*Gambusia affinis*	7	Top-water omnivores	Sand	Daniels & Felley (1992)
Fundulidae	*Fundulus olivaceus*	77	Top-water omnivores	Sand debris	Thomerson & Wooldridge (1970)
Centrarchidae	*Ambloplites ariommus*	52	Crayfish–fish	Sand debris	Johnson *et al.* (2010)
	Lepomis macrochirus	10	Generalist omnivores	Sand debris	Flemer & Woolcott (1966), Mullan & Applegate (1967)
	Lepomis megalotis	87	Generalist omnivores	Sand debris	Mullan & Applegate (1967)
	Lepomis miniatus	9	Generalist omnivores	Sand	VanderKooy *et al.* (2000)
	Micropterus punctulatus	9	Crayfish–fish	Sand debris	Scalet (1977)
Percidae	*Ammocrypta beanii*	52	Benthic invertivores	Sand	Heins (1985)
	Etheostoma histrio	15	Benthic invertivores	Sand debris	Kuhajda & Warren (1989)
	Etheostoma lynceum	2	N/A	N/A	N/A
	Etheostoma stigmaeum	3	N/A	N/A	N/A
	Etheostoma swaini	8	Benthic invertivores	Sand debris	Ruple *et al.* (1984)
	Percina nigrofasciata	69	Benthic invertivores	Sand debris	Mathur (1973)

N/A, data not available.

dietary categories were created based on feeding preference and preferred depth in the water column: benthic invertivores, generalist invertivores (generalist meaning the species has no preferred feeding depth in the water column), top-water omnivores, generalist omnivores, benthic omnivores, crayfish–fish and benthic detritus.

Canonical variate analysis (CVA) was used to identify distinct body shape features that best distinguish each trophic guild category and substratum category. Consensus shapes for each trophic guild were created and used to create thin-plate splines in MorphoJ (Klingenberg, 2011). Thin-plate splines were used to map the shape deformations in morphospace among each of the trophic groups. Consensus shapes of each species were made in tpsRelw (Rohlf, 2005). Discriminant analysis was used to examine whether the known groups of trophic guilds or substrata were distinct. Trophic guild and substratum categories were used as classifier variables for the discriminant analysis. CVA and discriminant analysis were carried out in MorphoJ.

PHYLOGENETIC ANALYSES

Mitochondrial (mt)DNA sequences (12s) were obtained from GenBank (*c*. 950 bp; www.ncbi.nlm.nih.gov/Genbank) or from sequencing species not previously available. GenBank data were obtained for the following species (Table II): *Ammocrypta beanii* Jordan 1877, *Cyprinella venusta* Girard 1856, *Lepomis macrochirus* Rafinesque 1819, *Percina nigrofasciata* (Agassiz 1854), *Gambusia affinis* (Baird & Girard 1853), *Fundulus olivaceus* (Storer 1845) and *Hybopsis winchelli* Girard 1856. For the following 10 species, DNA was extracted from fin clips using the Qiagen Dneasy Blood and Tissue kit (www.qiagen.com) following the manufacturer's instructions: *Ambloplites ariommus* Viosca 1936, *Etheostoma swaini* (Jordan, 1884), *Etheostoma histrio* Jordan & Gilbert 1887, *Lepomis megalotis* (Rafinesque 1820), *Lepomis miniatus* (Jordan 1877), *Notropis longirostris* (Hay 1881), *Notropis texanus* (Girard 1856), *Micropterus punctulatus* (Rafinesque 1819), *Noturus leptacanthus* Jordan 1877 and *Pimephales vigilax* (Baird & Girard 1853). The *12s* gene was amplified *via* PCR with the primers Phea and Tval (Kocher *et al*., 1989; Springer *et al*., 1995). PCRs consisted of 2·50 μl of 10× PCR buffer, 0·75 μl of $MgCl_2$, 0·50 μl of deoxynucleotide triphosphate (dNTP; 10 mM), 0·50 μl of each primer (10 mM), 0·25 μl of Taq polymerase, 0·50 μl of genomic DNA and the remaining volume consisting of H_2O for a total of 5 μl. The amplification profile was as follows: an initial denaturation at 94° C for 1 min, then 30 cycles at 94° C for 40 s, annealing at 52° C for 45 s, 72° C for 1 min and a final extension at 72° C for 5 min. All PCR products were visualized on a 0·8% agarose gel to assess quality. The *12s* gene was then sequenced with the forward primer 12s and reverse L1901 primer (Kocher *et al*., 1989; Springer *et al*., 1995).

Alignment and editing of sequences was performed in Sequencher 4.6 (www.genecodes.com). Loops in the *12s* gene, which can cause ambiguous alignment, were excluded from the analysis (Wang & Lee, 2002). Maximum parsimony analysis of the *12s* gene was performed using phylogenetic analysis using parsimony *(PAUP*) using unweight heuristic search (Swofford, 2000), simple search and tree bisection and reconnection (TBR) branch swapping for species where five or more individuals were collected during field sampling. Multiple equally parsimonious trees were found, and a strict consensus tree was made.

COMPARATIVE ANALYSES

The influences of phylogeny, trophic guild and substratum type on body shape were tested with three separate simple Mantel tests using distance matrices of body shape, trophic guild, phylogenetic and substratum type (Mantel & Valand, 1970). The body shape distance matrix was created using Procrustes distances among species pairs from a canonical variate analysis (CVA) in MorphoJ. The substratum data matrix was obtained by ln transforming the abundance data for every species in each substratum type, and then creating a Bray–Curtis resemblance matrix. The trophic resemblance matrix was constructed by attributing 1 to a pair of species that share a trophic guild and 0 was given to species pairs that do not share a trophic guild. Three-way Mantel tests were used to test the null hypothesis that the body shapes of the fish assemblage are independent of substratum, branch length distance and trophic guild. A three-way Mantel test was used to test the correlation between a matrix of Procrustes distances and matrices of substratum data, phylogenetic data and trophic resemblance. The Mantel tests were run in R

TABLE II. The specimen Southeastern Louisiana University Vertebrate Museum collection number (SLU Cat. #), tissue number collection number (TC #), and GenBank reference number of the *12s* ribosomal RNA gene used for all species collected from 2011 to 2012

Family	Species	SLU Cat. #	SLU TC #	GenBank #
Cyprinidae	*Cyprinella venusta*	8507, 8535, 8552, 8572	N/A	AF023187.1
	Hybopsis winchelli	8510, 8551, 8569	N/A	AF148343.1
	Luxilus chrysocephalus	8573	N/A	N/A
	Notropis longirostris	8522, 8532, 8550, 8571	TUMNH 199460	KM370983
	Notropis texanus	8541, 8570	TUMNH 199447	KM370984
	Opsopoeodus emiliae	8538	N/A	N/A
	Pimephales vigilax	8539	TC-SLU 4733	KM370986
Ictaluridae	*Noturus leptacanthus*	8513, 8527, 8542, 8565	TC-SLU 1724	KM370989
	Noturus miurus	8537	N/A	N/A
	Ictalurus puntulatus	8566	N/A	N/A
Catostomidae	*Hypentelium nigricans*	8525, 8555	N/A	N/A
	Moxostoma poecilurum	8523, 8556	N/A	N/A
Atherinopsidae	*Labidesthes sicculus*	8512	N/A	N/A
Poeciliidae	*Gambusia affinis*	8511, 8568	N/A	AP004422.1
Fundulidae	*Fundulus olivaceus*	8508, 8536, 8553, 8574	N/A	AP006776.1
Centrarchidae	*Ambloplites ariommus*	8518, 8540, 8557	TC-SLU 2312	KM370985
	Lepomis macrochirus	8515, 8549, 8558	N/A	NC_015984.2
	Lepomis megalotis	8516, 8534, 8554, 8559	TC-SLU 1738	KM370981
	Lepomis miniatus	8514, 8526, 8560	TC-SLU 1745	KM370987
	Micropterus punctulatus	8516, 8524, 8564	TC-SLU 1743	KM370980
Percidae	*Ammocrypta beanii*	8520, 8530, 8548, 8562	N/A	AY372765.1
	Etheostoma histrio	8546, 8561	TC-SLU 1738	KM370988
	Etheostoma lynceum	8545	N/A	N/A
	Etheostoma swaini	8519, 8529, 8543	TC-SLU 02.079	KM370982
	Etheostoma stigmaeum	8531, 8544	N/A	N/A
	Percina nigrofasciata	8521, 8533, 8547, 8563	N/A	AY372802.1

N/A, data not available.

with 9999 random matrix permutations using the vegan package in R (Oksanen *et al.*, 2010; R Development Core Team; www.r-project.org).

After assessing the phylogenetic signal using MorphoJ, a phylogenetic generalized least-square (pGLS) analysis and partial Mantel tests (Smouse *et al.*, 1986; Martins & Hansen, 1997) were used to determine whether the relationship between body shape and ecological or dietary data is an artefact of phylogenetic relationship among species. pGLS is a powerful method for exploring the covariance among traits while taking into account the non-independence of taxa due to shared evolutionary history (Martins & Hansen, 1997). pGLS is expressed as $y = \beta x + \varepsilon$, where y is the aligned consensus shape co-ordinates for each species, x is the matrix of average ecological data, β is the vector of partial regression coefficients and ε the error term. pGLS allows the error term to have a variance–covariance matrix where the covariance between each taxa pair is derived from the branch lengths of the phylogeny giving the relationships of the taxa. This variance–covariance matrix was calculated assuming a Brownian model of evolution, where the covariance between any pair of traits decreases linearly over time (as branch length increases) since their divergence. All transformation functions were left at 1·0, leaving the model to run as expected under the Brownian model (Martins & Hansen, 1997). In this study, the aligned consensus shapes of species with five or more individuals were regressed against the main effects of average flow and the average depth with the phylogenetic relationship taken into account using pGLS, testing the null hypothesis of no covariance between the matrix shape data and distance matrices of substratum and trophic data while accounting for phylogenetic relationships. A one-tailed ANOVA test was used to determine the relative importance of each ecological trait. pGLS was carried out in R using the caper package (R Development Core Team; Orme *et al.*, 2012).

In this study, two partial Mantel tests were used to test for a correlation between Procrustes distance matrix and trophic guild matrix as well as Procrustes distance matrix and substratum matrix while controlling for the phylogenetic relationships. In this way, the influence of phylogeny was removed from the comparison of the Procrustes distance matrix to the trophic guild and substratum matrices. The partial Mantel tests were run in R with 9999 random matrix permutations using the vegan package (Oksanen *et al.*, 2010; R Development Core Team).

To explore the patterns of covariance of body shape and ecological data for each species without controlling for phylogenetic relationships, a two-block partial least-square (PLS) analysis was performed in MorphoJ. PLS analysis is used to examine a pattern of covariance, if any exists, between two blocks or sets of variables (Rohlf & Corti, 2000). The *P*-value was estimated from 9999 random permutations. The ecological data were ln transformed for both PLS and pGLS.

Finally, the phylogenetic tree was superimposed into morphospace defined by the co-ordinates of the first two principal components. This was done in MorphoJ, which calculates the hypothetical ancestral shape of each species at each node using square-change parsimony weighted by branch length (Maddison, 1991). The phylogeny is then superimposed into morphospace according to the reconstructed ancestral values. The permutation test for phylogenetic signal was also used to test the null hypothesis of no phylogenetic structure in the data (Klingenberg & Gidaszewski, 2010).

RESULTS

SPECIMENS

Twenty-six species consisting of 580 specimens across seven families were collected from 2011 to 2012 in the Tickfaw River (Table II). The average flow and depths for all species across all seasons are shown in Table III.

PHYLOGENETIC ANALYSIS

Ten species were successfully sequenced for the 12s ribosomal RNA gene (mtDNA) and combined to data collected from GenBank. The data set consisted of 1036 bp, 652 variable characteristics and 256 parsimony informative characteristics. The parsimony analysis was limited to taxa for which five or more individuals were collected during the ecological sampling. Two equal parsimonious trees were recovered and both trees are similar in topologies and generally match the evolutionary relationships from previous studies. The first tree was randomly chosen for this portion of the analysis. Monophyletic groups are recovered for the Centrarchidae, Cyprinidae and Percidae (Fig. 2).

TABLE III. Number of individuals, mean ± S.E., minimum (min) and maximum (max) stream flow and depth for each species collected for all seasons (2011–2012)

Species	Number	Flow ($cm\,s^{-1}$)			Depth (cm)		
		Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max
Ambloplites ariommus	16	16·172 ± 2·643	2·250	35·000	42·688 ± 4·026	15·000	63·000
Ammocrypta beanii	52	23·663 ± 1·464	0·000	46·000	41·644 ± 2·151	10·500	107·000
Cyprinella venusta	109	18·314 ± 1·047	0·000	45·000	55·995 ± 2·516	13·000	118·000
Etheostoma histrio	15	23·450 ± 3·149	0·000	50·500	48·967 ± 5·634	13·000	90·500
Etheostoma lynceum	2	27·500 ± 18·500	9·000	46·000	44·250 ± 5·250	39·000	49·500
Etheostoma stigmaeum	3	16·500 ± 15·003	1·000	46·500	34·167 ± 6·002	26·500	46·000
Etheostoma swaini	8	30·563 ± 5·639	4·000	50·500	41·750 ± 4·934	25·000	64·000
Fundulus olivaceus	77	11·455 ± 1·375	0·000	50·500	35·156 ± 1·652	8·500	76·000
Gambusia affinis	7	11·071 ± 3·308	1·000	26·500	26·786 ± 6·612	10·500	57·500
Hybopsis winchelli	22	4·568 ± 1·445	0·000	24·000	45·250 ± 2·106	38·000	79·500
Hypentelium nigricans	3	25·667 ± 4·969	19·500	35·500	47·333 ± 15·836	16·000	67·000
Ictalurus punctatus	2	19·500 ± 15·500	4·000	35·000	33·500 ± 6·500	27·000	40·000
Labidesthes sicculus	4	10·250 ± 3·982	1·500	17·000	35·000 ± 4·907	23·500	47·500
Lepomis macrochirus	10	13·050 ± 2·748	5·000	31·000	33·200 ± 5·605	15·000	73·000
Lepomis megalotis	87	10·448 ± 1·077	0·000	48·000	43·925 ± 2·284	1·000	112·000
Lepomis miniatus	9	10·500 ± 2·821	0·000	18·500	31·111 ± 4·839	17·500	57·500
Luxilus chrysocephalus	1	25·000	25·000	25·000	58·000	58·000	58·000
Micropterus punctulatus	9	12·333 ± 3·379	0·000	32·500	38·667 ± 6·518	15·000	63·000
Moxostoma poecilurum	3	15·667 ± 5·333	5·000	21·000	15·000 ± 0·000	15·000	15·000
Notropis longirostris	44	24·216 ± 1·767	3·000	45·000	35·664 ± 2·384	15·000	79·500
Notropis texanus	5	24·875 ± 4·862	14·000	37·500	44·125 ± 7·119	16·000	64·000
Noturus leptacanthus	8	31·25 ± 5·270	14·000	50·500	43·950 ± 8·819	16·000	112·000
Noturus miurus	1	28·500	28·500	28·500	59·000	59·000	59·000
Opsopoeodus emiliae	2	0·000	0·000	0·000	38·000	38·000	38·000
Percina nigrofasciata	69	22·275 ± 1·515	1·500	37·123	37·630 ± 1·910	1·000	80·000
Pimephales vigilax	8	5·813 ± 2·952	0·000	35·125	35·125 ± 8·267	22·500	46·000

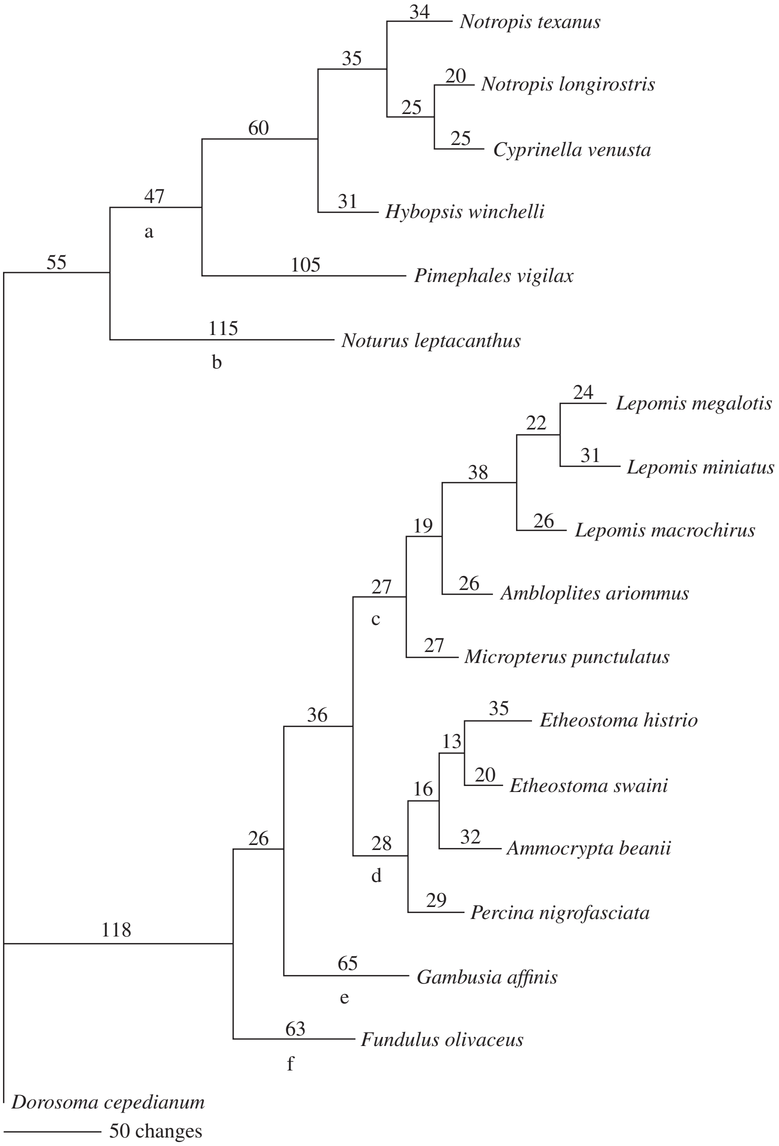

FIG. 2. A tree from a maximum parsimony analysis of the *12s* mitochondrial RNA gene of all specimens with five or more specimens collected from 2011 to 2012, with the branch lengths shown. Each clade is labelled by family: Cyprinidae (a), Ictaluridae (b), Centrarchidae (c), Percidae (d), Poeciliidae (e) and Fundulidae (f).

GEOMETRIC MORPHOMETRIC ANALYSES

Body shape

The first two axes of the shape PCA account for 79·7% of the total variance, where the first axis (49·1% of variance) describes the changes in the dorsal-fin and pelvic-fin placement, and the variance in the second axis (30·7% of variance) is explained

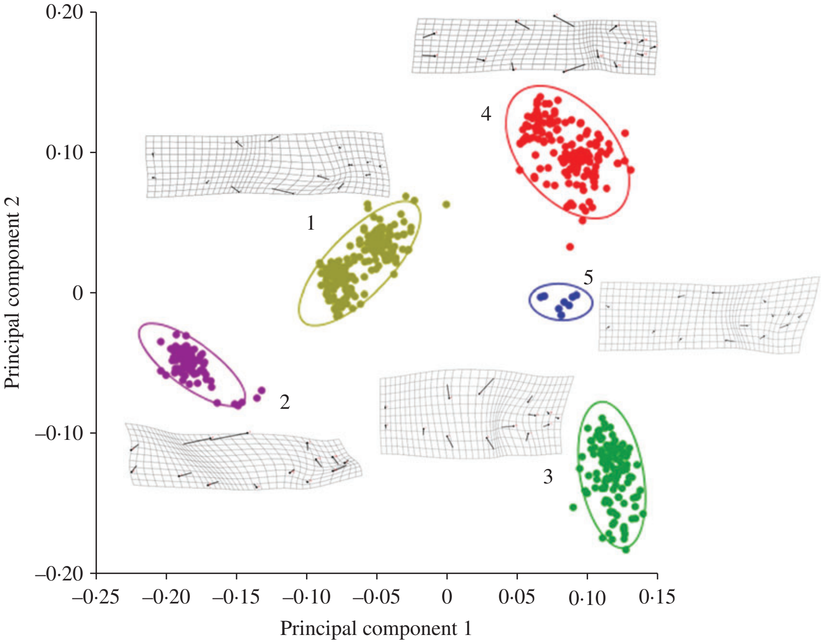

FIG. 3. Principal component analysis of all species from Tickfaw River (Lake Pontchartrain basin), with 95% confidence ellipses and consensus body shapes for each ecomorphotype: 1, generalist ecomorphotype (●); 2, top-water ecomorphotype (●); 3, structure-oriented ecomorphotype (●); 4, benthic ecomorphotype (●); 5, roaming-predator ecomorphotype (●). The first axis describes the changes in the dorsal-fin and pelvic-fin placement, and the variance in the second axis explains the compression in body shape dorso-ventrally.

by a compression in body shape dorso-ventrally. PCA reveals five distinct natural groupings (Fig. 3). Species in group 1 (generalist ecomorphotypes), exhibiting a rounded-fusiform body shape, are in the centre of the morphospace. These include minnows (Cyprinidae), suckers (Catostomidae) and a silverside (Atherinopsidae): *C. venusta*, *H. winchelli*, *Hypentelium nigricans* (LeSueur 1817), *Labidesthes sicculus* (Cope 1865), *Luxilus chrysocephalus* Rafinesque 1820, *Moxostoma poecilurum* Jordan 1877, *Opsopoeodus emiliae* Hay 1881, *N. longirostris*, *N. texanus* and *P. vigilax*. Within this group, species tend to have a relative position along a gradient in morphospace based on their feeding position in the water column, where the species on the right of group 1 have an inferior mouths and the species on the left tend have terminal mouths. Group 2 (top-water ecomorphotype) (Poeciliidae and Fundulidae) is characterized by having a posteriorly placed dorsal fin, depressed head, a slightly upturned head and superior mouth. Members of this group include *G. affinis* and *F. olivaceus*. Group 3, the structure-oriented group, consisting solely of centrarchids, includes *L. megalotis*, *L. macrochirus*, *L. miniatus* and *A. ariommus*, has a gibbose body shape, a wide head, a posteriorly placed pectoral fin, a large mouth and large eyes. Group 4, the benthic ecomorphotype (Fig. 3), is characterized by having dorso-ventrally compressed body shape, posteriorly placed anal and pelvic fins, dorsally placed eyes, inferior to sub-terminal mouth placement and posteriorly placed pectoral fins. Group 4 includes darters (Percidae) and a catfish (Ictaluridae): *A. beanii*, *E. histrio*, *Etheostoma lynceum* Hay 1885, *Etheostoma stigmaeum* (Jordan 1877), *E. swaini*,

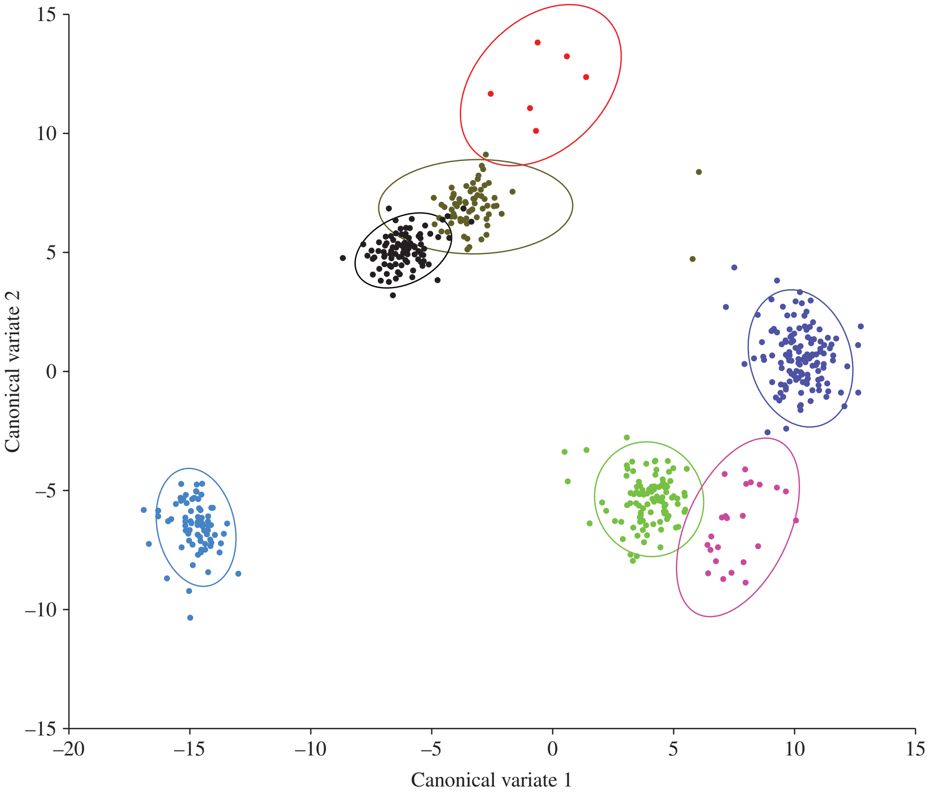

FIG. 4. Canonical variate analysis of the shape data for all species collected from the Tickfaw River (Lake Pontchartrain basin) using trophic guild categories as the *a priori* classifiers, with 95% confidence ellipses for each trophic group. The placement of the dorsal and pelvic fins explains much of the variation in the first axis, and the variation in the second axis describes the compression in body shape dorso-ventrally. ●, Benthic detritus; ●, benthic invertivores; ●, benthic omnivores; ●, crayfish–fish; ●, generalist invertivore; ●, generalist omnivore; ●, top-water omnivore.

Ictalurus punctatus (Rafinesque 1818), *N. leptacanthus*, *Noturus miurus* Jordan 1877 and *P. nigrofasciata*. Finally, group 5 (roaming-predator ecomorphotype) consists of solely *M. punctulatus* (Centrarchidae). Although related to the members of group 3, *M. punctulatus* is found in its own unique morphospace defined by having a fusiform-like body shape, a large head, large eyes, large mouth and anteriorly placed dorsal and pelvic fins.

Trophic guilds

The first two axes of the CVA account for 86·9% of the variation (Fig. 4), where the placement of the dorsal and pelvic fins explains the variation in the first axis, and the variation in the second axis describes the compression in body shape dorso-ventrally. Using trophic guild as an *a priori* grouping variable (Table I), the CVA identifies distinct body shape groupings for each trophic category (Figs 4 and 5). Although there is an overlap in laterally compressed body shapes between crayfish-fish feeders and generalist invertivores in the CVA, the crayfish-fish feeders tend to have a larger,

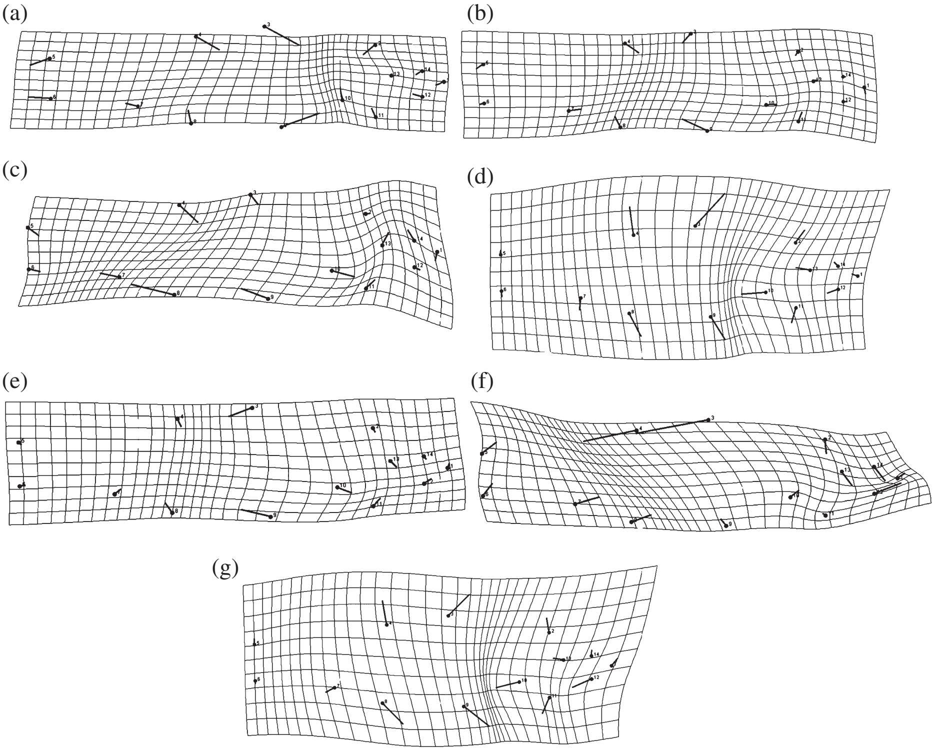

Fig. 5. Consensus body shapes of each trophic guild: (a) benthic invertivore, (b) benthic omnivore, (c) benthic detritus, (d) generalist invertivore, (e) generalist omnivore, (f) top-water omnivore and (g) crayfish–fish.

slightly upturned mouth when the average configurations of the trophic guilds, as projected in deformation grids, are examined [Fig. 5(d), (g)]. The generalist omnivores and benthic omnivores also partially overlap with a fusiform body shape, but the benthic species have a more inferior mouth and dorso-ventrally compressed body shape [Fig. 5(b), (e)]. The benthic invertivores are characterized by dorso-ventrally compressed body shape and posteriorly placed dorsal and pelvic fins. Detritus feeding species tend to have an anteriorly set anal and pelvic fin, as well as an inferior mouth and dorso-ventrally compressed fusiform body shape [Fig. 5(c)]. Top-water feeders are characterized by having an upturned head and posteriorly placed dorsal fin [Fig. 5(f)].

The discriminant analysis predicted all trophic guild pair-wise comparisons classifications correctly, with the exception of one individual in two comparisons: benthic omnivores *v*. benthic invertivores and benthic omnivores *v*. generalist omnivores (Table IV). Both the CVA and discriminant analysis point to each trophic guild having distinct body shapes. No distinct body shape was found for any substratum type by CVA.

COMPARATIVE ANALYSES

The Mantel tests reveal significant correlation between the matrix of Procrustes distances (pair-wise distances in morphospace) to pair-wise branch lengths ($r = 0{\cdot}413$,

Table IV. Discriminant analysis classification–misclassification table of the comparison between trophic guilds. Analysis 1 was between benthic omnivores (BO) and generalist omnivores (GO). Analysis 2 was between benthic omnivores (BO) and benthic invertivores (BI)

Analysis 1	BO	GO	Total	Analysis 2	BO	BI	Total
BO	76	0	76	BO	75	1	76
GO	1	112	113	BI	0	151	151

Table V. One-way, partial and three-way Mantel tests comparing morphological distances (Procustes distances) with the substratum, trophic and branch length distances of all species (with five or more individuals collected in total)

Comparison	*r*-Value	*P*-value
Simple Mantel test		
Shape *v.* substratum	0·143	>0·05
Shape *v.* trophic	0·554	<0·001
Shape *v.* phylogeny	0·413	<0·001
Phylogeny *v.* trophic	0·336	<0·001
Partial Mantel test		
Shape *v.* trophic with partial correlation on phylogeny	0·484	<0·001
Shape *v.* substratum with partial correlation on phylogeny	0·219	>0·05
Three-way Mantel test		
Shape *v.* trophic + phylogeny	0·601	<0·01

$P < 0{\cdot}001$) and to trophic guild ($r = 0{\cdot}554$, $P < 0{\cdot}001$), rejecting the null hypothesis of the distance matrix of shape data being independent of the distance matrices of branch length distance and trophic guild data. This suggests that the morphological structure of this assemblage is correlated with both trophic and phylogenetic structures. No correlation was found between the Procrustes distances and substratum type ($r = 0{\cdot}143$, $P > 0{\cdot}05$). After controlling for possible phylogenetic constraint using the partial Mantel test, the correlation between trophic guild and morphological distance dropped ($r = 0{\cdot}484$, $P < 0{\cdot}001$), and the correlation between substratum and morphological distance remain non-significant ($r = 0{\cdot}219$, $P > 0{\cdot}05$). The three-way Mantel test explains 36% of the variance that was an increase in the r^2 value, from 0·306 to 0·361, when compared with the simple Mantel tests (Table V).

PLS analysis reveal a positive correlation between body shape and flow without controlling for phylogeny ($r = 0{\cdot}361$, $P < 0{\cdot}01$). There is also a correlation between body shape and water depth ($r = 0{\cdot}266$, $P < 0{\cdot}05$). Species with fusiform and compressed body shapes tend to be found in areas of higher flow and depth (Table III).

pGLS reveal a correlation between body shape and flow ($r^2 = 0{\cdot}244$, $P < 0{\cdot}05$), rejecting the null hypothesis of no covariance between the matrix shape data and distance matrices of substrate and trophic data. Stream depth is not a significant predictor of body shape. The pGLS model of $x = \text{depth} + \text{flow}$ was found to be significant ($r^2 = 0{\cdot}350$, $P < 0{\cdot}05$), where x is the Procustes co-ordinates of all species with five or more individuals collected. The regression of ecological characteristics using pGLS

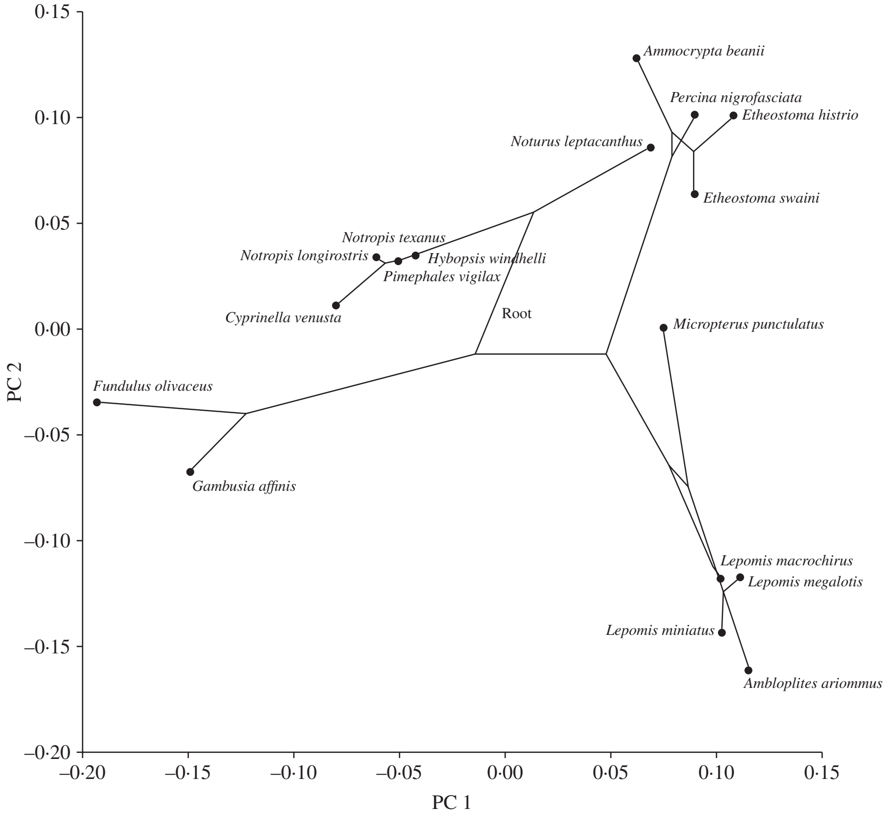

FIG. 6. The molecular phylogenetic tree of species with five or more individuals collected superimposed onto morphospace, where PC1 describes the changes in the dorsal-fin and pelvic-fin placement, and the variance in the PC2 is explained by a compression in body shape dorso-ventrally.

indicates that flow has the strongest influence on body shape ($F_{1,1} = 4{\cdot}888$, $P < 0{\cdot}05$) in the model.

The null hypothesis of no phylogenetic signal within the body shape data is rejected ($P < 0{\cdot}001$). The tree overlaid onto morphospace shows a strong phylogenetic signal within the body shape data. Species tend to group together morphologically within the same family, although some exceptions to this general trend are evident (Fig. 6). Both *N. leptacanthus* and *M. punctulatus* appear to be more morphologically similar to the darters than to their congenerics. The Cyprinidae is recovered in the centre of the PCA, closest to the tree root; the Cyprinidae have a similar shape to the hypothetical ancestral body shape according to the ancestral node.

DISCUSSION

TROPHIC GUILD AND BODY SHAPE

The first objective of this study was to test for correlation of body shape and trophic guilds of the species in the Tickfaw River fish assemblage, with and without the influence of evolutionary history. The relationship between diet and morphology has been widely debated. Some studies suggest that morphology is strongly correlated with diet (Gatz, 1979*b*; Winemiller *et al.*, 1995; Wagner *et al.*, 2009; Cochran-Biederman & Winemiller, 2010), whereas others have found weak or non-existent relationships (Grossman, 1986; Douglas & Matthews, 1992; Winemiller & Adite, 1997). A strong relationship between body shape and trophic guild was found (Fig. 4 and Table V), even after controlling for phylogenetic relationships among these species (Table V and Fig. 6). The body shape data have a strong phylogenetic signal and, therefore, cannot be considered independent (Felsenstein, 1985). The strong correlation between phylogenetic distance and morphological distance suggests that any correlation between morphology and trophic guild may be a result of a species' evolutionary history rather than adaptation to biological processes (Table V). The relationship between body shape and trophic guild, however, still remains after accounting for phylogenetic relationships (Table V). This indicates that the strong relationship between morphological and trophic guild is not solely due to phylogenetic relatedness. This implies, along with the results of the discriminant analysis, that measurement of body shape can be used as a valid predictor of trophic niche in this Gulf of Mexico coastal-plain river fish assemblage.

HABITAT AND BODY SHAPE

The second objective of this study was to test for a correlation of body shape and the habitat of the species in this fish assemblage, with and without the influence of phylogenetic relationships. The relationship between morphology and habitat has previously been addressed with mixed results (Chan, 2001; Herler, 2007; Oliveira *et al.*, 2010; Pease *et al.*, 2012). In this study, no correlation between body shape and substratum was recovered, but the substratum characterizations used may have been too coarse to detect any relationship. A significant correlation between body shape and flow exists, however, even after controlling for the phylogenetic influence, suggesting that body shape can be used to predict a stream flow regime for fish species in this Gulf of Mexico coastal-plain river. Water velocity is the most important variable in explaining the variance in body shape in the pGLS model. This result is not surprising because of the well-known relationship between shape and hydrodynamics in aquatic systems (Keast & Webb, 1966; Webb, 1984, 1988). What is more interesting are the low r^2 values of this relationship, with an r^2 value of 0·130 (PLS) and 0·265 (pGLS), respectively. The correlation between body shape and flow would be expected to be much stronger because the ability of a fish to move efficiently through water depends on its shape. The lower correlation values are probably caused by species such as *P. vigilax* and *H. winchelli*, which are found in lower flow than would be expected based on their fusiform shape alone. The fusiform shape allows species to maintain swimming speeds at a lower energy cost, but causes lower prey capture success in low-flow areas, giving these species a competitive disadvantage (Webb, 1984; Rincón *et al.*, 2007; Langerhans, 2008). This could be an instance where choice of habitat is not reflected by the phenotype of an organism, but instead is structured by other biological processes such as behaviour or availability of prey items. Behaviour has been shown to be more important in determining the diet selection of fish species than morphology (Grossman, 1986). It is plausible that the habitat selection of *P. vigilax* and *H. winchelli* is driven by behaviour rather than morphology and, if true, would be discordant with the ecomorphological hypothesis. The correlation between body shape and stream depth found by PLS analysis was weak and was probably due to the partial co-linearity shared between flow and depth. No relationship between body shape and stream depth was found using pGLS. These results imply that body shape of stream fishes cannot be predicted by stream depth. Although position in the water column was not tested in the study, other studies have shown that position in the water column is important in delineating resources within a fish assemblage (Dibble & Harrel, 2000) and could be partially dictated by body shape.

ECOMORPHOLOGICAL PATTERNS

Finally, the ecomorphological patterns of this Gulf of Mexico coastal-plain fish assemblage were explored. In addition, this study attempts to integrate the ecomorphological groupings (Fig. 3) with biological aspects pertaining to the species obtained during the study and from the literature, as proposed by Reilly & Wainwright (1994). An examination of the ecomorphological diversification patterns in the Tickfaw River reveals five distinct groups (Fig. 3). Each group was further examined through the integration of morphological characteristics with the function of these morphological characteristics and the ecology of the species within each group, as proposed by Reilly & Wainwright (1994).

First, a generalist ecomorphotype was recovered (Fig. 3, group 1) and consists of *H. nigricans*, *M. poecilurum*, *C. venusta*, *H. winchelli*, *L. chrysocephalus*, *N. longirostris*, *N. texanus*, *O. emiliae*, *P. vigilax* and *L. sicculus*. This group comprises species with a rounded-fusiform body shape. The rounded-fusiform body shape reduces water resistance, allowing species to have more effective propulsion and maintenance of velocity at a lower energy cost in aquatic systems, and suggests that these species reside in areas with moderate to high flow (Webb, 1984, 1988). In this study, species with a fusiform body shape are generally found in moderate to high flow; however, this is not always the case, as discussed for *P. vigilax* and *H. winchelli* above. The species within this generalist ecomorphotype group (Fig. 3, group 1) can be classified as either omnivore or detritivore, with their mouth position varying from terminal to inferior. The variability of the mouth position allows for optimal feeding at different positions within the water column. For example, *P. vigilax* has an inferior mouth that is adapted to feeding primarily off the bottom of the stream (Parker, 1964), whereas the terminal mouth of *C. venusta* is better adapted for feeding in the upper portion of the water column (Dibble & Harrel, 2000). In this study, species within this group fall along a gradient in morphospace based on their mouth position, suggesting different feeding preferences within the water column (Gatz, 1979*b*). Although this group has a well-defined body shape, no distinct set of ecological preferences can be defined for the group. The species in this group exploit a wide range of substratum types, flow regimes, depths and food items. The niche breadth of a species cannot be explained by ecomorphological analyses and is one weakness of such analyses (Ricklefs & Miles, 1994). It is troublesome, if not impossible, to define the ecomorphotype of the rounded-fusiform species due to the wide range of niches exploited by species within this group; therefore, the rounded-fusiform shape could be considered a generalist body shape. Interestingly, Oliveira *et al.* (2010) reported that species of fishes with a fusiform shape, or 'generalist body shape', and omnivorous diet are found in the centre of the morphospace, which is consistent with the findings of this study. It is possible that species fall out in morphospace with the ancestral species in the centre with more derived species at the periphery (Fig. 6). Cyprinidae and Catostomidae are morphologically similar in respect to body shape, although these are not sister families within the Cypriniformes (Saitoh *et al.*, 2006). The shared body shape between these families, however, cannot be attributed to either convergence or inherited phenotype from a common ancestor without including other families within the Cypriniformes.

Second, the top-water ecomorphotype group only contains two species, *F. olivaceus* and *G. affinis*. This group comprises species that feed primarily on terrestrial invertebrates, algae, detritus and other invertebrates (Thomerson & Wooldridge, 1970; Daniels & Felley, 1992). These species have a superior mouth that is often associated with surface feeding. The upturned mouth may also facilitate aquatic surface respiration in low oxygen environments (Lewis, 1970). The posteriorly placed pectoral fins suggest that species are adapted for bursts of speed but not for sustained movement (Keast & Webb, 1966; Webb, 1984). This is probably why areas of lower flow are preferred by these species (Table III). This group has a distinct niche that is associated with a unique body shape, allowing for a well-defined ecomorphotype. Fundulidae and Poeciliidae share similar morphological adaptations for the niches in which they reside, but are not sister taxon groups within the Cyprinodontiformes clade (Betancur *et al.*, 2013). Whether these adaptations were derived from a common ancestor or occur due to convergent evolution can only be answered with an in-depth study of the body shape evolution within the Cyprinodontiformes clade.

Third, a structure-oriented ecomorphotype group was also recovered and comprises centrarchids: *A. ariommus*, *L. macrochirus*, *L. megalotis* and *L. miniatus*. These species are commonly found in low-flow areas near submerged structures (Table III; Ross *et al.*, 1987). The gibbose body shape of fishes in this group is adapted for greater manoeuvrability, allowing them to perform quick, small angled manoeuvres (Keast & Webb, 1966; Webb, 1984; Helfman *et al.*, 2009). The species within this group are well suited for habitats with structural complexity and low flow. The group comprises insectivores that feed throughout the water column, as well as species that primarily feed on crayfishes or fishes (Table I). The gibbose body shape allows these species to more readily capture food items in these habitat types. When the ecomorphology of *A. ariommus* is compared with other members of this group, the predatory nature of this species is evident by the larger eyes, mouth and head. It is likely that the shared morphological characteristics of the group in question are derived from a common ancestor (Fig. 2; Near & Koppelman, 2009).

Fourth, the benthic ecomorphotype (Fig. 3, group 4) is the largest group consisting of percids and ictalurids: *A. beanii*, *E. histrio*, *E. lynceum*, *E. stigmaeum*, *E. swaini*, *I. punctatus*, *N. leptacanthus*, *N. miurus* and *P. nigrofasciata*. This group is categorized by benthic species with dorso-ventrally compressed body shapes; a body shape that is often characteristic of species that live in rapidly flowing currents (Keast & Webb, 1966; Gatz, 1979*b*; Webb, 1988). The members of group 4 typically occupy areas of moderately fast currents within the stream and in moderate depth (Table III), and have dorso-ventrally compressed body shapes. These species are subject to intense resistance force from drag and lift, also known as the Bernoulli effect (Webb, 1984), and are commonly found within riffles or runs. Some species of fishes, including the species listed above, overcome these forces by remaining in contact with the bottom substratum, thereby eliminating the water pressure beneath their body (Webb, 1988). The depressed body shape gives them a distinct advantage over other species in these habitat types. These species, with depressed body shape and inferior to sub-terminal mouth placement, are well adapted for their trophic guild (Keast & Webb, 1966; Webb, 1984; Helfman *et al.*, 2009), and their roles as benthic insectivores. The well-defined benthic ecomorphotype can be related to the niche of these species. This benthic ecomorphotype group is non-monophyletic, containing darters and a species of catfish. Darters (Perciformes) and madtoms (Siluriformes) are not phylogenetically related, yet the madtoms possess a similar morphology to the darters, allowing them to swim near the bottom in high-flow habitats (Table III) and search for benthic invertebrates.

Lastly, the roaming-predator ecomorphotype consists of a single species: *M. punctulatus*, which occupies a unique morphospace in spite of the phylogenetic relationship it shares with all the members of the structure oriented ecomorphotype (Fig. 6), and it appears to have diverged morphologically from its sister genus *Lepomis* (Near & Koppelman, 2009). *Micropterus punctulatus* has a more compressed fusiform shape than the other members in its family, suggesting that *M. punctulatus* would be found in higher flow areas when compared with the gibbose-shaped, structure-oriented ecomorphotype (Werner, 1977), but this is not the case. Instead, *M. punctulatus* resides in similar currents to the other Centrarchidae (Table III). Based on a body shape comparison, *M. punctulatus* would be less suited for quick, small angled manoeuvres that are optimal for residing in areas with structural complexity. The larger mouth, eye size and head size of *M. punctulatus* would indicate a predatory nature for this species (Gatz, 1979*b*), which is supported by gut analyses (Scalet, 1977). *Micropterus punctulatus* and *A. ariommus* have very similar diets, yet very different overall body shapes (Scalet, 1977; Johnson *et al.*, 2010). This suggests niche partitioning via habitat or feeding site preference.

GENERAL CONSIDERATIONS

Organisms can be considered products of their evolutionary history, which in turn is reflected in their morphology and behaviour (Douglas & Matthews, 1992; Ricklefs & Miles, 1994). Douglas & Matthews (1992) argue that ecomorphological patterns are products of evolutionary history rather than adaptation to biological processes or convergence, thereby restricting ecomorphological studies to within families. Even though the influence of phylogeny is strong in this study, the ecomorphological patterns observed in this Gulf of Mexico coastal-plain fish assemblage are not solely derived from phylogenetic history, instead being partially due to adaptation to biological processes. This implies the validity of ecomorphological analyses as a tool to predict the ecological attributes of species and to evaluate general patterns of assemblage structure, although cases are seen where ecomorphology may be misleading. Instances where habitat choice, in this case flow regime, does not reflect morphology are also an area of concern for ecomorphological studies. Ecologists should be cautious when making assumptions about assemblage structure and the ecologies of organisms when using ecomorphological analyses, and should always employ phylogeny as a null hypothesis when working with distantly related species. The ecology of organisms is not wholly determined by their phenotype, but rather by multiple processes. As body shape can be linked with trophic and habitat components of fish species, this would imply that body shape is associated with both α and β niches (Ackerly & Cornwell, 2007). Therefore, body shape may give species an advantage in certain habitats, as well as limit the amount of trophic overlap of species within that habitat type, exhibiting characteristics of both environmental filtering and the principle of limiting similarity (MacArthur & Levins, 1967; Ingram & Shurin, 2009).

In this study, geometric morphometrics is a helpful tool for ecomorphological studies, demonstrating a relationship between phenotype and niche position even across distantly related species. No relationship between body shape and substratum was found in this study. Body shape can be used to predict trophic and flow regimes even after phylogenetic relationships were taken into account, and therefore could be used as a surrogate for certain niche dimensions and assemblage structures of species of fishes in a Gulf of Mexico coastal-plain river. Ecomorphology has been shown to be a powerful tool in ecological studies, but biologists should always be mindful of historical or current constraints when using this type of analysis.

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