SYMPOSIUM

Pharyngeal Jaws Converge by Similar Means, Not to Similar Ends, When Minnows (Cypriniformes: Leuciscidae) Adapt to New Dietary Niches

Kelsie M. Pos,[1],[*] Stacy C. Farina,[†] Matthew A. Kolmann[‡] and Nicholas J. Gidmark[*]

[*]Department of Biology, Knox College, Galesburg, IL, USA; [†]Department of Biology, Howard University, Washington, DC, USA; [‡]Department of Biology, George Washington University, Washington, DC, USA

[1]E-mail: kmpos@knox.edu

Synopsis Convergent evolution is at the forefront of many form-function studies. There are many examples of multiple independent lineages evolving a similar morphology in response to similar functional demands, providing a framework for testing hypotheses of form-function evolution. However, there are numerous clades with underappreciated convergence, in which there is a perceived homogeneity in morphology. In these groups, it can be difficult to investigate causal relationships of form and function (e.g., diet influencing the evolution of jaw morphology) without the ability to disentangle phylogenetic signal from convergence. Leuciscids (Cypriniformes: Leuciscidae; formerly nested within Cyprinidae) are a species-rich clade of fishes that have diversified to occupy nearly every freshwater trophic niche, yet are considered to have relatively low morphological diversity relative to other large freshwater clades. Within the North American leuciscids, many genera contain at least one herbivore, insectivore, and larvaphage. We created 3D models from micro-computed tomography scans of 165 leuciscid species to measure functionally relevant traits within the pharyngeal jaws of these fishes. Using a published phylogeny, we tested these metrics for evolutionary integration, phylogenetic signal, and correlation with diet. Measurements of the pharyngeal jaws, muscle attachment areas, and teeth showed strong positive evolutionary correlation with each other and negative evolutionary correlation with measurements of the inter-ceratobranchial ligament (ICB ligament). Using diet data from published literature, we found extensive dietary convergence within Leuciscidae. The most common transitions we found were between herbivorous and invertivorous taxa and between insectivore types (aquatic vs. terrestrial). We document a trade-off in which herbivorous leuciscids have large teeth, short ICB ligaments, and large muscle attachment areas, whereas insectivorous leuciscids showed the opposite pattern. Inverse patterns of morphological integration between the ICB ligament the rest of the pharyngeal jaw correspond this dietary trade-off, which indicates that coordinated evolution of morphological traits contributes to functional diversity in this clade. However, these patterns only emerge in the context of phylogeny, meaning that the pharyngeal jaws of North American leuciscids converge by similar means (structural changes in response to dietary demands), but not necessarily to similar ends (absolute phenotype).

Introduction

Convergence is a common theme in evolutionary biology; similar phenotypes evolve repeatedly in response to similar selective pressures. In addition to natural selection, phylogenetic inertia imposes genetic, developmental, and structural constraints to shape an organism (Connell 1980; Barel et al. 1989).

Ecomorphological studies quantitatively link structure with function by using ecological parameters such as diet as a proxy for function (e.g., Norton 1991, 1995; Pineda-Munoz et al. 2016). These studies examine structure–function relationships on a macroevolutionary scale to understand how evolutionary processes shape structures across evolutionary history

(Lauder 1981). Evolutionary integration refers to the correlated evolution of traits across a clade, as well as shared functional demands (Marroig and Cheverud 2005; Monteiro and Nogueira 2010; Collar et al. 2014; Roberts, et al. 2018). While evolutionary eco-morphological studies can link evolutionary integration to ecology, this has only been cursorily explored (Monteiro and Nogueira 2010; Meloro et al. 2011; Collar et al. 2014). Shared functional demands have been shown to influence the tendency of structures to be evolutionarily integrated, often overcoming developmental constraints (Marroig and Cheverud 2005; Monteiro and Nogueira 2010; Meloro et al. 2011; Collar et al. 2014). One group that exemplifies form–function relationships in feeding morphology is the ray-finned fishes (Actinopterygii), a group of 30,000 species that have evolved myriad, disparate feeding ecologies and morphologies (Baliga and Law 2016; Evans et al. 2017; Buser et al. 2017; Hernandez et al. 2018; Hundt and Simons 2018).

One of the most striking aspects of fish trophic anatomy is the presence in some groups of pharyngeal jaws, a secondary set of jaws in the back of the throat (Wainwright 1989; German et al. 2009; Simons and Gidmark 2010; Pasco-Viel et al. 2010; Gidmark et al. 2014; Ahnelt et al. 2015; Gidmark et al. 2015; Leung et al. 2017). The oral jaws of many fishes act alone for both prey capture and processing; however, in other fishes, the oral jaws act only as a means for prey capture, with prey processing and transport taking place entirely under the control of the pharyngeal jaws. Durophagous (crushing) pharyngeal jaws are famously considered to have contributed to morphological diversification in cichlids and labrid wrasses (Liem 1973; Kaufman and Liem 1982; Wainwright 2006). In these groups, the pharyngeal jaws are the primary structures for prey processing, which has allowed the oral jaws to become more specialized for prey capture. The functional "decoupling" of prey capture and processing may have allowed the diversity of trophic morphology to increase, thereby also increasing dietary breadth within these groups (Liem 1973; Wainwright 2006).

Cypriniforms have a modified posterior-most gill arch, the hypertrophied fifth ceratobranchial (CB V), which acts as pharyngeal jaws (Sibbing 1982; Simons and Gidmark 2010). These jaws are actuated by a muscular sling to mechanically process prey against the base of the skull. The pharyngeal jaw bones (Hulsey 2006; Zeng and Liu 2011), teeth (German et al. 2009; Gidmark et al. 2014; Gidmark et al. 2015), and musculature (Eastman 1971) are known to differ with dietary specialization, and many of these characteristics persist throughout phylogenetic history (Rüber and Adams 2001; Burress 2015). Cypriniformes represent a radiation of fishes with extreme pharyngeal diversity relative to cichlids and are far older (90+ vs. 60 myr, respectively; Hughes et al. 2018). The order Cypriniformes includes the family Leuciscidae (formerly nested within Cyprinidae) that accounts for around 20–30% of total freshwater fish diversity, and includes herbivores, piscivores, insectivores, and other dietary niches (Gee 1961; Vanicek and Kramer 1969; Tan and Armbruster 2018). The taxonomy of this group has been difficult to establish due to convergence (He et al. 2008), but recent efforts have further resolved the leuciscid phylogeny (Hollingsworth et al. 2013). Therefore, leuciscids have become a powerful system for examining convergence in phenotypic and ecological evolution (Eastman and Underhill 1973; Pasco-Viel et al. 2010). Here, we use North American leuciscids (*sensu* Tan and Armbruster 2018; formerly Leuciscinae and Phoxininae) as a model system for examining patterns of morphological convergence across trophic niches. Our objectives were to (1) describe relevant axes of functional variability in North American leuciscid morphology, (2) determine whether pharyngeal jaw morphology correlates with diet and these associations are a result of convergence or phylogenetic inertia and (3) test for evolutionary integration among functional measurements of the leuciscid pharyngeal jaw.

Methods

Taxon sampling and digital data acquisition

Formalin-fixed, ethanol-preserved specimens (species chosen based on representation in Hollingsworth et al. 2013) were borrowed from the following natural history collections: Arizona State University Natural History Collections, Bell Museum of Natural History (University of Minnesota), Burke Museum (University of Washington), The Field Museum (University of Chicago), Illinois Natural History Survey (University of Illinois), McClung Museum of Natural History and Culture (University of Tennessee), North Carolina Museum of Natural Sciences, Texas Natural History Collections, and Tulane University Biodiversity Research Institute University of Kansas Biodiversity Institute and Natural History Museum, University of Michigan Museum of Zoology. Specimens were micro computed tomography (CT) scanned at a resolution of 35 µm using the Bruker Skyscan 1173 (Bruker, Kontich) at the Karel F. Liem Imaging

Facility at Friday Harbor Laboratories (University of Washington, Friday Harbor, WA, USA).

Reconstructed scans were loaded into Fiji (Schindelin et al. 2012), where pharyngeal jaws were cropped (Crop3D add-on) and loaded into 3D Slicer (Fedorov et al. 2012; Kikinis et al. 2014), where thresholding levels were altered to maximize bone visibility. Pharyngeal jaws were then opened as 3D models in Geomagic Studio 2013 (3D Systems, Morrisville, NC, USA) where linear measurements were taken. Of our total dataset, 165 species are included in our evolutionary integration analyses and 102 are included in our dietary analyses. This paring down of species was due to limitations in scanning resolution, availability of dietary information, and because of the necessity of matching taxa to that of published phylogenetic hypotheses.

We analyzed nine anatomical metrics (Fig. 1) of the pharyngeal jaws to quantify the variation across species. Distance of the inter-ceratobranchial ligament (ICB ligament) between the pharyngeal jaws is termed "ligament length" (1), rostro-caudal distance is termed "ligament width" (2), and dorso-ventral distance is termed "ligament height" (3). Ligament height and width were multiplied to find ligament cross-sectional area. These metrics are a proxy for whether the ICB ligament acts as a stabilizer for the jaw (short and stout ligament) or as an element that maximizes relative motion between left and right pharyngeal jaw bones (long and skinny ligament). Next, we measured the distance from the base of the anterior-most tooth cusp to the posterior-end of the ICB ligament ("anterior arm length") (4) and "jaw broadness" (5) as the distance from the broadest, lateral-most point of the jaw to the anterior tip of jaw to measure elongation of the anterior arm toward the mouth of the fish. Greater elongation may allow for larger ligament surface area. We measured tooth length (6) and depth (7) from the tooth that displayed the clearest chewing surface and complete ankylosis. Tooth length was measured from the medial tip of the tooth to the base on the CB V, and depth was measured from the dorsal center of the chewing surface straight through to the ventral side of the tooth. We used tooth depth and length as a proxy for the amount of biomechanical processing done by the teeth. Then, we measured the area of the levator muscle attachment site (8) that included the area of the anterior and posterior side of the dorsal arm. Retractor muscle attachment area (9) was measured along the posterior side of the CB V. We used these attachment areas as proxies for muscle size (i.e., a larger levator attachment area site corresponds to a larger levator muscle). All data were
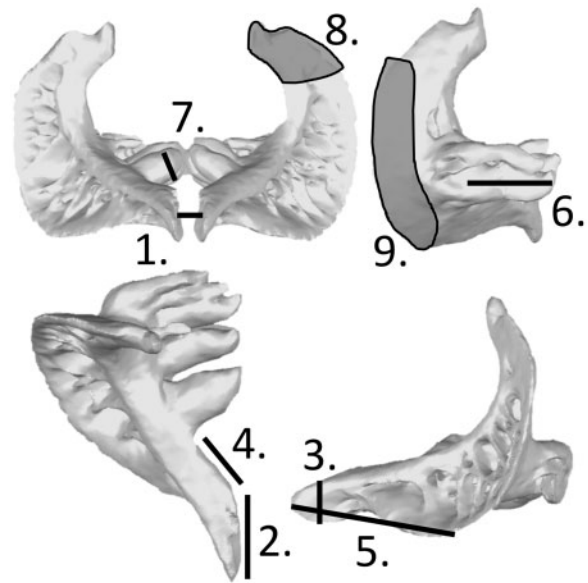

Fig. 1 Nine linear metrics measured from 3D models (from reconstructed Micro CT scans) of North American leuciscid pharyngeal jaws. Metrics include (1) Ligament length, (2) ligament width, (3) ligament height, (4) anterior-arm length, (5) jaw broadness, (6) tooth length, (7) tooth depth, (8) levator muscle attachment area, and (9) retractor muscle attachment area.

size corrected by dividing each measurement by the geometric mean of all nine metrics for each specimen.

Testing relationships among functional units and diet

Dietary data were extracted from the original species descriptions, published ecological manuscripts, select government agency or resource-management reports, FishBase (Froese and Pauly 2019), and other peer-reviewed articles detailing gut-contents of our species (Supplementary Table S2). However, leuciscid diets vary with geography, season, and ontogeny, and there was a high frequency of dietary overlap among largely omnivorous species. Rather than categorizing taxa into discrete dietary specializations, our method for exploring dietary convergence coded for the presence/absence of each prey type found within a particular species' diet. Since presence/absence data can be skewed by inclusion of rare events (in this case, rare prey), we chose to exclude prey which accounted for <5% of total prey volume or occurrence in gut content studies.

We annotated the phylogeny from Hollingsworth et al. (2013) with a matrix of diet data at the tips (Fig. 2) using *phytools* (Revell 2012). Using the character matrix of prey items as well as our anatomical metrics, we tested for relationships between morphology and diet by computing phylogenetic
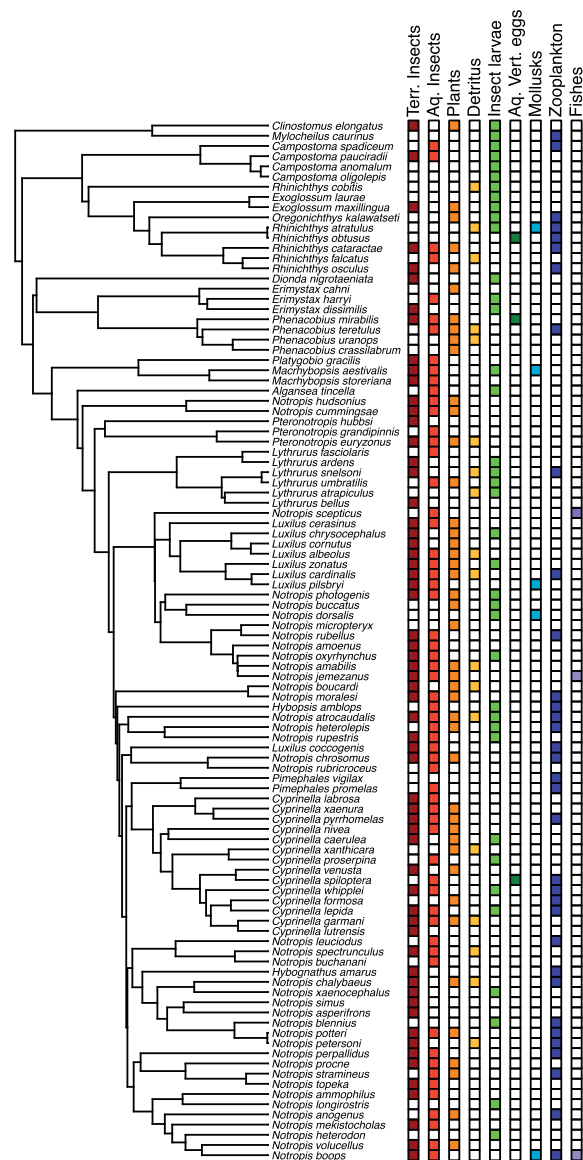
Fig. 2 Phylogenetic tree of North American leuciscids ([Hollingsworth et al. 2013](#)) with diet annotations. Most species feed on multiple prey types. Prey of this clade includes terrestrial insects (Terr. Insects), aquatic insects (Aq. Insects), plants, detritus, insect larvae, aquatic vertebrate eggs (Aq. Vert. eggs), mollusks, zooplankton, and fishes. Filled-in boxes denote the presence of a prey item in the diet of each species, and white boxes indicate an absence of a prey item reported in diet data.

ANOVAs using the *gls* function in the *nlme* package (Linear and Nonlinear Mixed Effects Models package) in R ([R Core Team 2016](#); [Pinheiro et al. 2018](#)), with a Brownian correlation structure using the *corBrownian* function in the *ape* package ([Paradis et al. 2004](#)). We conducted a principal component analysis using the *princomp* function ([R Core Team 2016](#)) and plotted principal components one and two (PC1 and PC2) on a phylomorphospace via the *phylomorphospace* function in the *phytools* package ([Sidlauskas 2008](#); [Revell 2012](#)).

Quantifying evolutionary integration

Our measurements define functionally relevant components (functional units) of the leuciscid pharyngeal jaw, including measurements of the CB V itself (jaw broadness and length of the anterior arm of the jaw), the teeth (length and depth), muscle attachment surface (levator and retractor muscles), and the ICB ligament (length, width, and height). Using size-corrected measurements, we quantified evolutionary integration among functional units using both phylogenetic generalized least squares (PGLS) models and correlations of phylogenetic independent contrasts (PICs) using a previously published phylogeny of leuciscids ([Hollingsworth et al. 2013](#)). We used PGLS to test for significance of covariance among functional units, and we used correlations of PICs to compare the strength of correlations among measurements. PGLS models were computed using the *gls* function in the *nlme* package (Linear and Nonlinear Mixed Effects Models package) in R ([R Core Team 2016](#); Pinheiro et al. 2018), with a Brownian correlation structure based on the [Hollingsworth et al. (2013)](#) phylogeny using the *corBrownian* function in the *ape* package ([Paradis et al. 2004](#)). PICs were computed using the *pic* function in the *ape* package ([Paradis et al. 2004](#)) and correlated using the *cor.origin* function in the PHYLOGR package ([Diaz-Uriarte and Garland 2014](#)). To confirm that these analyses were robust to differences in topology and branch lengths, we repeated these tests for evolutionary integration with a recently-published megaphylogeny of ray-finned fishes ([Rabosky et al. 2018](#)), pruned to only include the 139 leuciscid species that were included in both that tree and our dataset ([Supplementary Table S1](#)). Tests of phylogenetic signal (Blomberg's K and Pagel's λ) for each trait were implemented using the *phylosig* function (*geiger*; [Harmon et al. 2008](#)).

Evolutionary transitions among diet guilds

Our method for exploring dietary transitions assesses what prey types are found consistently across groups of taxa. In order to find common themes across largely omnivorous leuciscid taxa, our method creates synthetic diet categories from available gut content data by grouping leuciscid taxa by dietary similarity ([Egan et al. 2017](#), [2018](#); Egan JP and Buser TJ manuscript under review). After coding for the presence/absence of unique prey types found within a particular species' diet, we compiled a distance matrix, using Bray–Curtis dissimilarity, to represent diet divergences among leuciscid taxa. We

Table 1 Results from PGLS models and PIC correlations conducted using the Hollingsworth et al. (2013) phylogeny

	Tooth d	Tooth l	Ant. arm l	Jaw w	Levator aa	Retractor aa	Ligament l	Ligament h	Ligament w
Tooth d	1	0.0939	0.2193	0.0752***	0.2519***	0.2442***	−0.1427***	−0.2493*	−0.1747***
Tooth l	0.1435	1	0.1574	0.1747***	0.2906**	−0.0295	−0.0467	−0.6167***	0.1195*
Anterior arm l	0.0752	0.0824	1	0.2167***	0.4015*	0.3421*	−0.7160***	−1.521***	0.0733
Jaw w	0.3140*	0.4773**	0.3102*	1	0.1619***	0.0405	−0.1480	−1.849***	0.0705
Levator aa	0.3846*	0.2903*	0.2102	0.4427*	1	0.4317***	−0.1471*	−0.4981**	−0.1272*
Retractor aa	0.3281*	−0.0259	0.1576	0.1259	0.3800*	1	−0.1644**	−0.3964*	−0.3100***
Ligament l	−0.3141*	−0.0672	−0.5403***	−0.0780	−0.2120	−0.2693	1	−0.1538	0.4314***
Ligament h	−0.2041	−0.3304*	−0.4269*	−0.3628*	−0.2672	−0.2415	−0.0572	1	−0.0639*
Ligament w	−0.3915*	0.1752	0.0563	0.0379	−0.1867	−0.5169**	0.4394*	−0.1748	1

Values on the top right of the matrix are coefficients from PGLS models, representing the covariation between traits. Values on the bottom left are coefficients from correlations of phylogenetically independent contrasts, representing the strength of correlation between traits. Significance is indicated as follows: *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$. aa, attachment area; d, depth; h, height; l, length; w, width.

used the *hclust* function in R to perform a hierarchical cluster analysis, which iteratively joins individual clusters (according to proportion of prey items shared among leuciscid taxa, termed hereafter as diet "profiles"). According to their diet similarity, taxa were clustered using *hclust*'s "averaging" agglomeration method (i.e., UPGMA) (package vegan; Legendre and Legendre 2012). We then used an arbitrary 60% dissimilarity threshold like previous studies (Buchheister and Latour 2015; Egan et al. 2017; Hundt and Simons 2018), to determine how many of these clusters would be suitable to partition the diverse dietary profiles within our sample. We also wanted to examine the frequency of transitions to and from herbivorous, piscivorous, and insectivorous feeding modes; therefore, we ran a second set of analyses which coded leuciscids as belonging to any of these three categories if they consumed any manner of plants and detritus, fish, or insect or insect larvae, respectively. Finally, we used stochastic character mapping (Bollback 2006) to determine the directionality and frequency of evolutionary transitions among different dietary guilds. We iterated these simulations 1000 times across the concatenated phylogeny from Hollingsworth et al. (2013) using the "ARD" or all-rates different setting in *phytools* (Revell 2012).

Results

Measurements of the pharyngeal jaws (jaw broadness and length of the anterior arm), the teeth (length and depth), and muscle attachment surface (levator and retractor muscles) tended to be positively correlated with each other, but negatively correlated with the ligament (length, width, and height; Table 1). Figure 3 shows the strength and pattern on the correlations among functional measurements. We observed extensive convergence in the diet (Fig. 2) and we tied this diet variation to morphological measurements using phylogenetic ANOVA models (Table 2). Our phylomorphospace showed that plant eaters overlap nearly completely in morphology with non-herbivorous leuciscids (Fig. 4). Diverse trophic profiles arise convergently throughout the tree, with nearly every pair of sister taxa feeding on a different set of prey types.

Transitions between diet categories

Clustering algorithms and the 60% dissimilarity threshold supported five dietary "guilds," synthesized from the unique prey profiles of 102 leuciscid species: (1) herbivores, (2) aquatic invertivores, (3) aquatic insectivores, (4) insect larvaphagy, and (5) general omnivores (Fig. 4). These guilds overlapped considerably; for example, some manner of insect-feeding occurred in 92% of the taxa surveyed. Therefore, when we discuss dietary guilds, we acknowledge that some degree of insectivory occurs in most taxa, either incidentally or intentionally. Herbivores (4.9% of surveyed taxa) consumed exclusively plants and detritus. Aquatic invertivores (8.8% of taxa) ate both aquatic insects and non-insect arthropods like acarids, arachnids, isopods, water mites (Hydrocarina), and cladocerans. Aquatic insectivores (5.8%) consume mayflies, water beetles (Hydrophilidae), and backswimmers (Notonectidae), whereas insect larvaphages (1/3 of all surveyed taxa) fed on caddisfly, odonate, stonefly, and chironomid larvae. Finally, omnivorous leuciscids (45% of all taxa) fed on a variety of prey, including both terrestrial and aquatic insects (e.g., coleopterans and formicids), crustaceans like cladocerans, plants, detritus, annelid worms, and sometimes fish. Transitions from invertivory to herbivory, and the reverse were
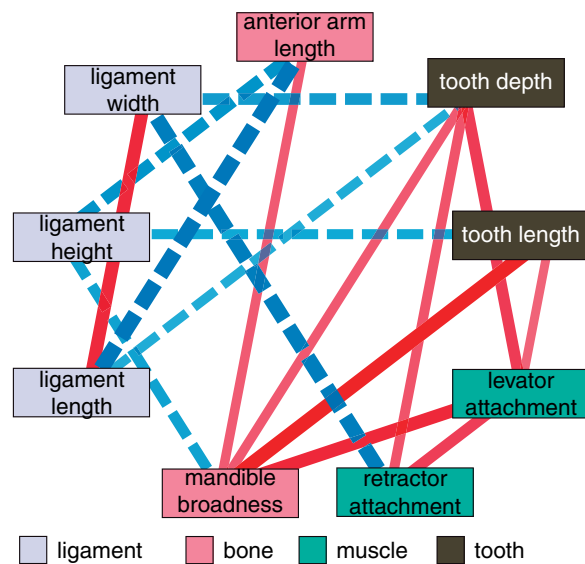
Fig. 3 Correlation map of functional metrics. Significant correlations of PICs are represented by lines connecting functional metrics, showing the extent of evolutionary integration among structures. Solid lines represent positive correlation, and dotted lines represent negative correlation. The thickness of the line is proportional to the strength of the correlation, based on correlation coefficients reported in Table 1.
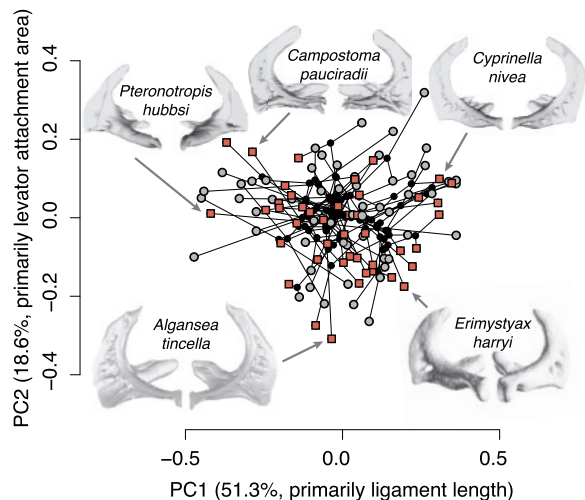

Fig. 4 Phylomorphospace of herbivorous and non-herbivorous leuciscids. The first two PC axes from our principal component analysis are plotted to show that herbivorous leuciscids (squares) have a nearly complete overlap in morphospace with leuciscids that do not have plants in their diets (open circles). Phylogenetic relationships among taxa are depicted by the lines connected to nodes (filled circles) to demonstrate the extent of convergence of morphology, with distantly related taxa occupying similar regions of morphospace.

the most common (Fig. 4) in this group. Those transitions were nearly twice as frequent as transitions between omnivory and aquatic insectivory, or larvaphagy and omnivory. Transitions from herbivory to omnivory were rare, whereas transitions among insectivorous guilds (omnivores, aquatic insect specialists, and larvaphages) were the most frequent and persistent. Piscivory was rare in our dataset, with only 3.6% of taxa ever feeding on fishes.

Discussion

We found extreme convergence in the diets of North American leuciscids (Fig. 2) with no genera uniformly allied with a singular trophic niche. We also found substantial convergence in morphology, with many anatomical measurements showing a lack of phylogenetic signal. We investigated the connection between dietary and morphological convergence, and we identified a trade-off in morphology between herbivorous leuciscids and insectivorous leuciscids. The evolution of herbivory was associated with convergent evolution of higher muscle attachment surface area, larger teeth, and a small ICB ligament. Conversely, the evolution of aquatic insectivory was associated with a large ICB ligament, narrow pharyngeal jaws, smaller teeth, and lower muscle attachment surface area. This functional trade-off was associated with patterns of evolutionary integration: measurements of the jaw, muscle attachment area, and teeth were positively correlated with each other and negatively correlated with measurements of the ICB ligament. We found evolutionary transitions between diets to be common within this group, with a particularly high number of transitions out of herbivory, which is a rarity in other groups of fishes.

Functional tradeoffs

Evolution of pharyngeal jaw morphology has many functional consequences for leuciscids, given the diversity of prey that they consume. Insect cuticle made of chitin and the cellulose-laden tissues of plants must be sheered apart by their consumers (Reilly et al. 2001; Vincent and Wegst 2004). The cell walls of plant material require herbivorous fishes to grind their food with high forces, which necessitates jaw stability (Reilly et al. 2001; Gidmark et al. 2014). The evolution of herbivory is associated with deeper teeth, a shorter ICB ligament, and larger levator muscle attachment surface for grinding this tough material, all of which contribute to high stability and force transmission (Gidmark et al. 2014) (Table 2). The chitinous exoskeletons of insects parallel these biomechanical demands but require insectivorous fishes to manipulate the pharyngeal jaw to both pierce hard prey and allow passage of prey to

Table 2 Tests for associations between diet and morphological measurements and estimates of phylogenetic signal

Measurement	Phylogenetic ANOVA model(s)	F of models	P	Pagel's λ	P	Blomberg's K	P
Tooth depth	Y ~ Plants	9.337	0.0029	0.21	ns	0.18	ns
	Y ~ -(Aq. Insects)	69.221	<0.0001				
Tooth length	Y ~ Zooplankton	9.066	0.0033	0.23	ns	0.20	ns
Tooth cusp to ligament	Y ~ Terrestrial Insects	15.540	<0.0001	0.19	0.04	0.27	0.003
	Y ~ -(Zooplankton)	9.103	0.0032				
Jaw broadness	Y ~ -(Aq. Insects)	17.275	<0.0001	0.42	<0.01	0.33	0.001
Levator attachment area	Y ~ Detritus	13.064	<0.0001	0.30	0.005	0.22	0.050
	Y ~ Plants	6.472	0.0125				
	Y ~ -(Aq. Insects)	32.1444	<0.0001				
Retractor attachment area	Y ~ Plants	41.757	<0.0001	0.35	<0.01	0.13	ns
	Y ~ -(Aq. Insects)	78.13271	<0.0001				
Ligament length	Y ~ Aq. Insects	25.629	<0.0001	0.47	0.02	0.20	0.01
	Y ~ -(Plant)	7.625	0.0069				
Ligament height	Y ~ -(Detritus)	7.449	0.0075	0.31	0.005	0.21	ns
	Y ~ Aq. Insects	5.407	0.0221				
Ligament width	Y ~ Aq. Insects	126.407	<0.0001	0.11	ns	0.09	ns
	Y ~ -(Plant)	76.341	<0.0001				
Ligament CSA	Y ~ Aq. Insects	23.532	<0.0001	0.22	ns	0.21	ns
	Y ~ -(Plant)	6.193	0.0145				
	Y ~ -(Terr. Insects)	6.329	0.0135				

Diets include plants, aquatic insects (Aq. Insects), detritus, zooplankton, and terrestrial insects (Terr. Insects). for ns $P > 0.05$.

the esophagus (Herrel and Aerts 2004). The evolution of insectivory is associated with the elongation of the ICB ligament and minimization of tooth and muscle attachment size to facilitate these biomechanical challenges (Table 2). This pattern is limited to leuciscids that eat aquatic insects, which tend to be small and easy to manipulate, relative to terrestrial insects. These associations between diet and morphology emerge when tested in the context of phylogeny, demonstrating that morphology has evolved in response to dietary demands, despite the extensive convergence seen in the pharyngeal jaws of leuciscids. The pharyngeal jaws of leuciscids converge by similar means, but not necessarily to similar ends. By this, we mean that leuciscid pharyngeal jaw morphology does not converge onto similar absolute phenotypes ("similar ends") based on diet, which is clearly demonstrated by the observation that herbivores occupy nearly the entire morphospace of leuciscid pharyngeal jaws. However, when phylogeny is taken into account, we see a clear evolutionary tendency for herbivores to have smaller ligaments, larger teeth, and larger muscle attachment surface areas ("similar means"). By identifying the pattern of morphological change in the pharyngeal jaws associated

with diet, the trophic profile of a given species potentially could be identified.

Integration

The pharyngeal jaw morphology of herbivorous and insectivorous leuciscids reflects the inverse morphological relationship between metrics of the ICB ligament and the rest of the pharyngeal jaw. The functional metrics of the pharyngeal jaws can be grouped into four types: muscle attachment areas (muscle), tooth dimensions (tooth), pharyngeal jaw dimensions (bone), and ICB ligament dimensions (ligament). The muscle, bone, and tooth measurements show evolutionary integration with positive covariation, and these structures tend to be larger in herbivores and smaller in insectivores, when phylogeny is taken into account (Fig. 3). Ligament measurements show an inverse relationship with the muscle, bone, and tooth metrics, both in terms of the direction of evolutionary integration (i.e., as one gets smaller, one gets larger) and the dietary associations. The evolutionary integration seen among these structures is likely due to a combination of genetic pleiotropy and developmental constraint (Klingenberg 2008), but the association with

diet indicates that natural selection also plays a considerable role in the coordinated evolution of these traits. Although high evolutionary integration has been shown to limit morphological evolution in some systems (Collar et al. 2014), it has also been suggested that natural selection can act on integrated traits to drive them to peripheral extents of the possible morphospace (Parsons et al. 2011). Coordinated evolution can also maintain traits that are not linked by pleiotropic effects, when shared functional demands require evolutionary integration (Marroig and Cheverud 2005; Monteiro and Nogueira 2010; Felice et al. 2018; Roberts et al. 2018). In this study, we have observed a trade-off in conflicting functional demands for herbivory and insectivory that likely plays a role in maintaining integration and disintegration within this highly morphologically and trophically convergent system.

To further examine the extent to which this evolutionary trade-off is maintained by functional demands rather than phylogenetic inertia, we quantified phylogenetic signal for each functional metric. Ligament and tooth traits appear to be evolving independently of phylogeny (i.e., non-significant K values), while features of the CB V (broadness and anterior arm length) show strong correlations with phylogeny ($\lambda < 0.5$). While it is tempting to infer that perhaps pharyngeal jaw bone traits are under strong constraint (phylogenetic conservatism) and ligaments are perhaps more labile, with ligaments responding more readily to functional demands, these generalizations can be misleading (Revell et al. 2008). However, a lack of phylogenetic signal in some metrics indicates that patterns of similarity in pharyngeal jaw morphology are not due solely to phylogenetic conservatism but also due to other evolutionary processes, such as natural selection, act to shape the pharyngeal jaws. The frequency of convergence in this clade and the high species richness makes North American leuciscids an ideal system for fitting evolutionary models to phenotypic data, allowing us to test for the different evolutionary processes at play in generating convergent phenotypes (Revell et al. 2008; Keck et al. 2016).

Dietary transitions

Herbivory and omnivory have been found to be evolutionary dead-ends for many vertebrate taxa (Davis et al. 2012; Price et al. 2012; Burin et al. 2016; Egan et al. 2018); however, herbivorous and omnivorous diet profiles were some of the most evolutionary labile in our dataset (Fig. 5). Omnivory may provide enough ecological flexibility for leuciscids to
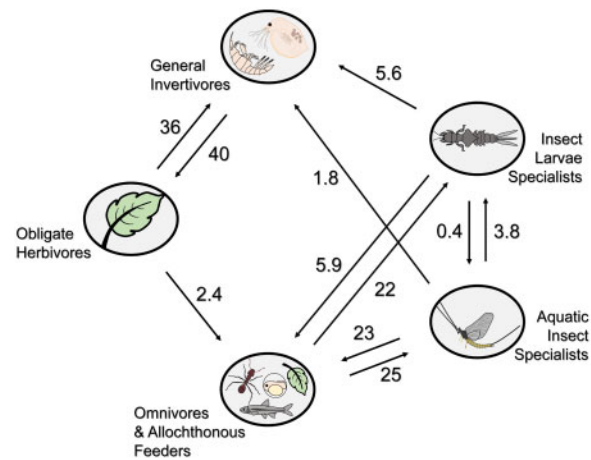

Fig. 5 Dietary transitions of North American leuciscids between herbivory, aquatic invertivory (zooplankton), aquatic insectivory, insect larvaphagy, and general omnivory. Arrows denote the direction of transition and numbers signify the frequency of transitions (single individuals).

capitalize on seasonally fluctuating prey resources. Correspondingly, some degree of insect-feeding was found in 92% of the taxa in our study, a resource subsidy that may aid in mitigating seasonal flux in resource availability or even macroevolutionary shifts in diet preference over larger timescales (Mundahl and Wissing 1987; Behrens and Lafferty 2007). Transitions between exclusive insectivory and either herbivory or invertivory were rare in our dataset, which suggests a biomechanical constraint when feeding predominantly on either plants or insects (a trade-off between stability and mobility). Mechanical processing is a rate-limiting step for consuming stubborn prey materials like cellulose (Day et al. 2011) and chitinous insect cuticle (Fänge et al. 1979; Evans and Sanson 2005). This is particularly important for leuciscids, because of simple gut morphology (German 2009; German et al. 2010) and lack of either oral jaw teeth or a stomach (Simons and Gidmark 2010). More natural history research into ontogenetic and seasonal diet variation, and how this pertains to feeding anatomy, is needed to broaden our understanding of leuciscid ecomorphology.

Conclusion

The ecomorphological diversity that leuciscids exhibit can be difficult to link to specific functional and ecological traits such as diet, given the extreme convergence in both diet and morphology. We have shown that analyzing diet in a phylogenetic context gives rise to clear patterns of dietary evolution and demonstrates conflicting functional demands for herbivory and insectivory. Inverse patterns of

morphological integration correspond with these conflicting demands, which indicate that coordinated evolution of morphological traits is a key mechanism for producing functional diversity in this clade. Evolutionary integration among pharyngeal jaw traits has also likely played a role in facilitating the many dietary transitions seen in leuciscids, providing the evolutionary lability to respond to the changes demands of diet.

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Supplementary data

Supplementary data available at *ICB* online.

References

Ahnelt H, Herdina AN, Metscher BD. 2015. Unusual pharyngeal dentition in the African Chedrin fishes (Teleostei: Cyprinidae): significance for phylogeny and character evolution. Zool Anz 255:85–102.

Baliga VB, Law CJ. 2016. Cleaners among wrasses: phylogenetics and evolutionary patterns of cleaning behavior within Labridae. Mol Phylogenet Evol 94:424–35.

Barel CD, Anker GC, Witte F, Hoogerhoud RJ, Goldschmidt T. 1989. Constructional constraint and its ecomorphological implications. Acta Morphol Neerl Scand 27:83–109.

Behrens MD, Lafferty KD. 2007. Temperature and diet effects on omnivorous fish performance: implications for the latitudinal diversity gradient in herbivorous fishes. Can J Fish Aquat Sci 64:867–73.

Bollback JP. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7:88.

Buchheister A, Latour RJ. 2015. Diets and trophic-guild structure of a diverse fish assemblage in Chesapeake Bay, U.S.A. J Fish Biol 86:967–992.

Burin G, Kissling WD, Guimarães PR Jr, Şekercioğlu ÇH, Quental TB. 2016. Omnivory in birds is a macroevolutionary sink. Nat Commun 7:11250.

Burress ED. 2015. Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. Hydrobiologia 748:7–27.

Buser TJ, Burns MD, López JA. 2017. Littorally adaptive? Testing the link between habitat, morphology, and reproduction in the intertidal sculpin subfamily Oligocottinae (Pisces: Cottoidea). PeerJ 5:e3634.

Collar DC, Wainwright PC, Alfaro ME, Revell LJ, Mehta RS. 2014. A novel feeding mode disrupts evolutionary integration of the skull in eels. Nat Commun 5:5505.

Connell JH. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35:131–8.

Day RD, German DP, Tibbetts IR. 2011. Why can't young fish eat plants? Neither digestive enzymes nor gut development preclude herbivory in the young of a stomachless marine herbivorous fish. Comp Biochem Physiol B Biochem Mol Biol 158:23–9.

Davis AM, Unmack PJ, Pusey BJ, Johnson JB, Pearson RG. 2012. Marine–freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae). J Evol Biol 25:1163–79.

Diaz-Uriarte R, Garland T. 2014. PHYLOGR: functions for phylogenetically based statistical analyses. R package version 1.0.8 (http://CRAN.R-project.org/package=PHYLOGR).

Eastman JT, Underhill JC. 1973. Intraspecific variation in the pharyngeal tooth formulae of some Cyprinid fishes. Copeia 1973:45–53.

Eastman JT. 1971. The pharyngeal bone musculature of the carp, *Cyprinus carpio*. J Morphol 134:131–40.

Egan JP, Chew US, Kuo CH, Villarroel-Diaz V, Hundt PH, Iwinski NG, Hammer MP, Simons AM. 2017. Diets and trophic guilds of small fishes from coastal marine habitats in western Taiwan. J Fish Biol 91:331–45.

Egan JP, Bloom DD, Kuo CH, Hammer MP, Tongnunui P, Iglésias SP, Sheaves M, Grudpan C, Simons AM. 2018. Phylogenetic analysis of trophic niche evolution reveals a latitudinal herbivory gradient in Clupeoidei (herrings, anchovies, and allies). Mol Phylogenet Evol 124:151–61.

Evans AR, Sanson GD. 2005. Biomechanical properties of insects in relation to insectivory: cuticle thickness as an indicator of insect 'hardness' and 'intractability'. Aust J Zool 53:9–19.

Evans KM, Waltz B, Tagliacollo V, Chakrabarty P, Albert JS. 2017. Why the short face? Developmental disintegration of the neurocranium drives convergent evolution in neotropical electric fishes. Ecol Evol 7:1783–801.

Fänge R, Lundblad G, Lind J, Slettengren K. 1979. Chitinolytic enzymes in the digestive system of marine fishes. Mar Biol 53:317–21.

Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin J-C, Pujol S, Bauer C, Jennings D, Fennessy FM, Sonka M, et al. 2012. 3D slicer as an image computing platform for the quantitative imaging network. Magn Reson Imaging 30:1323–41.

Felice RN, Randau M, Goswami A. 2018. A fly in a tube: macroevolutionary expectations for integrated phenotypes. Evolution 72:2580–94.

Froese R, Pauly D, (eds.). 2019. FishBase. World Wide Web electronic publication. (www.fishbase.org) (accessed online February 2019).

Gee JH. 1961. Ecology of the leopard dace *Rhinichthys falcatus* and its ecological relationships with the longnose dace *Rhinichthys cataractae* [UBC Theses and Dissertations]. University of British Columbia, Vancouver BC, Canada.

German DP, Neuberger DT, Callahan MN, Lizardo NR, Evans DH. 2010. Feast to famine: the effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). Comp Biochem Physiol A Mol Integr Physiol 155:281–93.

German DP, Nagle BC, Villeda JM, Ruiz AM, Thomson AW, Balderas SC, Evans DH. 2009. Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effects on gut size and digestive physiology. Physiol Biochem Zool 83:1–18.

Gidmark NJ, Taylor C, LoPresti E, Brainerd E. 2015. Functional morphology of durophagy in black carp, *Mylogpharyngodon piceus*. J Morphol 276:

Gidmark NJ, Tarrant JC, Brainerd EL. 2014. Convergence in morphology and masticatory function between the pharyngeal jaws of grass carp, *Ctenopharyngodon idella*, and oral jaws of amniote herbivores. J Exp Biol 217:1925–32.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–31.

He S, Mayden RL, Wang X, Wang W, Tang KL, Chen W, Chen Y. 2008. Family Cyprinidae (Actinopterygii: Cypriniformes) as evidenced by sequence variation in the first intron of S7 ribosomal protein-coding gene. Mol Phylogenet Evol 46:818–29.

Hernandez LP, Adriaens D, Martin CH, Wainwright PC, Masschaele B, Dierick M. 2018. Building trophic specializations that result in substantial niche partitioning within a young adaptive radiation. J Anat 232:173–85.

Herrel A, Aerts P. 2004. Biomechanical studies of food and diet selection. Nature Encyclopedia of Life Sciences. London: Nature Publishing Group.

Hollingsworth PR, Simons AM, Fordyce JA, Hulsey CD. 2013. Explosive diversification following a benthic to pelagic shift in freshwater fishes. BMC Evol Biol 13:272.

Hughes LC, Ortí G, Huang Y, Sun Y, Baldwin CC, Thompson AW, Arcila D, Betancur-R R, Li C, Becker L, et al. 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. Proc Natl Acad Sci U S A 115:6249–54.

Hulsey CD. 2006. Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw. Proc Biol Sci 273:669–75.

Hundt PJ, Simons AM. 2018. Extreme dentition does not prevent diet and tooth diversification within combtooth blennies (Ovalentaria: Blenniidae). Evolution 72:930–43.

Kaufman LS, Liem KF. 1982. Fishes of the suborder Labroidei (Pisces: Perciformes): phylogeny, ecology, and evolutionary significance. Breviora 472:1–19.

Keck F, Rimet F, Bouchez A, Franc A. 2016. phylosignal: an R package to measure, test, and explore the phylogenetic signal. Ecol Evol 6:2774–80.

Kikinis R, Pieper SD, Vosburgh K. 2014. 3D Slicer: a platform for subject-specific image analysis, visualization, and clinical support. In: Ferenc AJ, editor. Intraoperative imaging image-guided therapy. New York: Springer, Vol. 3. p. 277–89 (https://www.slicer.org).

Klingenberg CP. 2008. Morphological integration and developmental modularity. Annu Rev Ecol Evol Syst 39:115–32.

Legendre P, Legendre LFJ. 2012. Numerical Ecology Volume 24 of Developments in Environmental Modelling. Oxford, UK: Elsevier.

Leung C, Duclos KK, Grünbaum T, Cloutier R, Angers B. 2017. Asymmetry in dentition and shape of pharyngeal arches in the clonal fish Chrosomus eos-neogaeus: phenotypic plasticity and developmental instability. PLoS One. 12(4):1–19.

Lauder GV. 1981. Form and function: structural analysis in evolutionary morphology. Paleobiology 7:430–42.

Liem KF. 1973. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. Syst Zool 22:425–41.

Marroig G, Cheverud JM. 2005. Size as a line of least evolutionary resistance: diet and adaptive morphological radiation in New World monkeys. Evolution 59:1128–42.

Meloro C, Raia P, Carotenuto F, Cobb SN. 2011. Phylogenetic signal, function and integration in the subunits of the carnivoran mandible. Evol Biol 38:465–75.

Monteiro LR, Nogueira MR. 2010. Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures. Evolution 64:724–44.

Mundahl ND, Wissing TE. 1987. Nutritional importance of detritivory in the growth and condition of gizzard shad in an Ohio reservoir. Environ Biol Fishes 20:129–42.

Norton SF. 1991. Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. Ecology 72:1807–19.

Norton SF. 1995. A functional approach to ecomorphological patterns of feeding in cottid fishes. Environ Biol Fishes 44:61–78.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–90.

Parsons KJ, Son YH, Albertson RC. 2011. Hybridization promotes evolvability in African cichlids: connections between transgressive segregation and phenotypic integration. Evol Biol 38:306–15.

Pasco-Viel E, Charles C, Chevret P, Semon M, Tafforeau P, Viriot L, Laudet V. 2010. Evolutionary trends of the pharyngeal dentition in Cypriniformes (Actinopterygii: Ostariophysi). PLoS One 5:6.

Pineda-Munoz S, Lazagabaster IA, Alroy J, Evans AR. 2016. Inferring diet from dental morphology in terrestrial mammals. Methods Ecol Evol 8:481–91.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2018. nlme: linear and nonlinear mixed effects models. R package version 3.1-137 I (https://CRAN.R-project.org/package=nlme).

Price SA, Hopkins SS, Smith KK, Roth VL. 2012. Tempo of trophic evolution and its impact on mammalian diversification. Proc Natl Acad Sci U S A 109:7008–12.

R Core Team. 2016. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing (https://www.R-project.org).

Rabosky DL, Chang J, Title PO, Cowman PF, Sallan L, Friedman M, Kaschner K, Garilao C, Near TJ, Coll M, et al. 2018. An inverse latitudinal gradient in speciation rate for marine fishes. Nature 559:392.

Reilly SM, McBrayer LD, White TD. 2001. Prey processing in amniotes: biomechanical and behavioral patterns of food reduction. Comp Biochem Physiol A Mol Integr Physiol 128:397–415.

Revell LJ, Harmon LJ, Collar DC. 2008. Phylogenetic signal, evolutionary process, and rate. Syst Biol 57:591–601.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3:217–23.

Roberts AS, Farina SC, Goforth RR, Gidmark NJ. 2018. Evolution of skeletal and muscular morphology within the functionally integrated lower jaw adduction system of sculpins and relatives (Cottoidei). Zoology 129:59–65.

Rüber L, Adams DC. 2001. Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. J Evol Biol 14:325–32.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, et al. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–82.

Sibbing FA. 1982. Pharyngeal mastication and food transport in the carp (*Cyprinus carpio* L.): a cineradiographic and electromyographic study. J Morphol 172:223–58.

Sidlauskas B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. Evolution 62:3135–56.

Simons AM, Gidmark NJ. 2010. Systematics and phylogenetic relationships of Cypriniformes. In T. Grande, editor. Gonorynchiformes and ostariophysan relationships – a comprehensive review. Enfield (CT): Science Publishers. p. 409–40.

Tan M, Armbruster JW. 2018. Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi). Zootaxa 4476:6–39.

Vanicek CD, Kramer RH. 1969. Life history of the Colorado squawfish, *Ptychocheilus lucius*, and the Colorado chub, *Gila robusta*, in the Green River in Dinosaur National Monument, 1964–1966. Trans Am Fish Soc 98:193–208.

Vincent JF, Wegst UG. 2004. Design and mechanical properties of insect cuticle. Arthropod Struct Dev 33:187–99.

Wainwright PC. 1989. Functional morphology of the pharyngeal jaw apparatus in Perciform fishes: an experimental analysis of the Haemulidae. J Morphol 200:231–45.

Wainwright PC. 2006. Functional morphology of the pharyngeal jaw apparatus. In: Shadwick RE, Lauder GV, editors. Fish physiology. Vol. 23. San Diego (CA): Elsevier Academic Press. p. 79–101.

Zeng Y, Liu H. 2011. The evolution of pharyngeal bones and teeth in Gobioninae fishes (Teleostei: Cyprinidae) analyzed with phylogenetic comparative methods. Hydrobiologia 664:183–97.