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True grit? Comparative anatomy and evolution of gizzards in fishes

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Abstract

Gut morphology frequently reflects the food organisms digest. Gizzards are organs of the gut found in archosaurs and fishes that mechanically reduce food to aid digestion. Gizzards are thought to compensate for edentulism and/or provide an advantage when consuming small, tough food items (e.g., phytoplankton and algae). It is unknown how widespread gizzards are in fishes and how similar these structures are among different lineages. Here, we investigate the distribution of gizzards across bony fishes to (1) survey different fishes for gizzard presence, (2) compare the histological structure of gizzards in three species, (3) estimate how often gizzards have evolved in fishes, and (4) explore whether anatomical and ecological traits like edentulism and microphagy predict gizzard presence. According to our analyses, gizzards are rare across bony fishes, evolving only six times in a broad taxonomic sampling of 51 species, and gizzard presence is not clearly correlated with factors like gut length or dentition. We find that gizzard morphology varies among the lineages where one is present, both macroscopically (presence of a crop) and microscopically (varying tissue types). We conclude that gizzards likely aid in the mechanical reduction of food in fishes that have lost an oral dentition in their evolutionary past; however, the relative scarcity of gizzards suggests they are just one of many possible solutions for processing tough, nutrient-poor food items. Gizzards have long been present in the evolutionary history of fishes, can be found in a wide variety of marine and freshwater clades, and likely have been overlooked in many taxa.

KEYWORDS
Actinopterygii, detritivory, gastrolith, microphagy, trituration

1 | INTRODUCTION

Food breakdown is essential for efficient nutrient absorption, which powers an organism's needs for growth, reproduction, and cellular repair (Goodman, 2010). Food reduction is composed of two basic processes: mechanical and chemical processing (Schwenk & Rubega, 2005). During mechanical processing, shear and compressive loading break food into smaller particles, increasing the surface area over which chemical digestion can occur (Kolmann et al., 2016; Lucas, 2004; Lucas & Luke, 1984; Reilly et al., 2001). This increases the efficiency of enzymatic activity and maximizes nutrient extraction. The processes of mechanical and chemical reduction are well-described in mammals, with both beginning in the mouth during mastication (Prinz & Lucas, 1997). However, another organ evolved for food reduction is the gizzard. Gizzards are a modification of the gut tract consisting of layers of hypertrophied smooth muscle that constrict a lumen filled with gastroliths (Schmitz & Baker, 1969; Svihus, 2011). There have been several definitions of 'gastrolith' since its first scientific use by Mayne (1854) (Wings, 2007); for our purpose, gastroliths are stones of various sizes that range from small pebbles to fine-grained sand that are ingested by the animal for the explicit purpose of mechanical reduction.

Gastroliths are ingested by the fish and are collected in the gizzard, where smooth muscle likely forces the gizzard to contract, trapping food between the triturating surfaces created by the gastroliths. The grit in gizzards generates shear on tough food particles, reducing particle size and thus maximizing the relative metabolic return on nutrient-deficient food items (Veiberg et al., 2007; Western & Ssemakula, 1982). The relationship between gizzard functional anatomy and diet is well described in birds, including the relationship between gastrolith size and prey type, the reducing power of differently sized gastroliths, and the forces generated by gizzards of different volumes (Moore, 1998b; Moore et al., 1998). Although gizzard function is best described in birds, gizzards are present in many clades of vertebrates, including other archosaurs. Gizzards were once thought to be synapomorphic for the entire Archosauria (Varricchio, 2001). However, the gizzards of crocodilians and birds are now thought to have evolved independently and be functionally analogous (Takasaki & Kobayashi, 2020). Of all the vertebrate groups that have been reported to have gizzards, the anatomical and ecological functions of gizzards in fishes are the least understood.

Fishes that are reported to have gizzards include some characiforms, mullet (Mugilidae), the aptly named gizzard shads (e.g., *Dorosoma* spp. and *Nematalosa* spp.), and several other clupeiforms, suckers (Catostomidae), angelfishes (Pomacanthidae), and surgeonfishes (Acanthuridae), among others (Castro et al., 1961; Chakrabarti & Ghosh, 2014; Jones, 1968; Konow & Bellwood, 2011; Rowntree, 1903; Schmitz & Baker, 1969; Spiegel et al., 2011; Thomson, 1954). There is some overlap in the dietary habits of these species – most are bottom-feeders that consume microscopic food items like diatoms, algae, and detritus (Choat et al., 2002; Egan et al., 2017; Konow & Bellwood, 2011). Ingestion of these food particles, which are too small to be sensed and eaten individually, is termed microphagy (Jørgensen, 1966; Sanderson & Wassersug, 1993). Microphagy is challenging given the need to aggregate food particles prior to and possibly during digestion. Moreover, there is an intense need for mechanical reduction of cellulose and silicate-laden foods like phytoplankton, algae, and plant matter, especially given the low nutrient density of these resources (Egan et al., 2018; Mundahl & Wissing, 1988).

Compared to maintaining both teeth and guts, gizzards may require less metabolic investment and be less prone to declining performance with age than teeth (Leigh et al., 2008). Indeed, many fishes that are reported to have gizzards have very small teeth or are edentulous and may use the gizzard and ingested gastroliths as a constantly renewable triturating surface. Alternatively, gizzards could be a case of contingency and constraint. When evolving a detritivorous or microphagous niche, edentulous organisms may find it easier to modify the gut tract than to dust off the genetic programming needed to generate a dentition capable of mechanically reducing tough food items (Fritz et al., 2011). In other words, the gizzard may be an example of contingency, in that gizzards arose because edentulism had already evolved, or of constraint, because teeth were difficult or impossible to reacquire. Finally, we propose that gizzards might be adapted for shearing apart incredibly small prey particles, like those found in detrital diets. Gizzards thereby avoid the minimum threshold of dentitions (Lucas, 2004), where large processing surfaces like teeth can only deform but not crack individual prey particles.

The relationship between the anatomical form and ecological function of gizzards in fishes is not yet clear. The presence of a gizzard in disparate lineages of fish lends an opportunity for exploring the ecological and anatomical correlates of gizzard presence in the most diverse clade of vertebrates, the bony fishes (Osteichthyes). We had several objectives: (1) survey different microphagous and non-microphagous fishes for gizzard presence; (2) compare the microstructural anatomy of gizzards in three species of fishes using histology; (3) estimate how often gizzards have evolved across the Fish Tree of Life (FToL); and (4) assess evidence for evolutionary correlations between gizzard presence, diet, and the occurrence of feeding structures like teeth and pharyngeal jaws using phylogenetic comparative methods. We predict that while not all microphagous fishes have gizzards, all fishes with gizzards will be microphagous – a situation arising from the absence of comparable mechanical processing tools (i.e., teeth) in some taxa vs. others. Likewise, we expect that many fishes with gizzards will have some form of reduced oral dentition or be entirely edentulous. Finally, we expect gizzard-bearing fishes to have a comparatively longer hindgut for digesting detritus and plant matter.

2 | METHODS

2.1 | Specimen acquisition, dissection, and morphometrics

To understand the similarities and differences among gizzard microstructures, we used histological sectioning and staining to visualize tissue types in three species. For histological sectioning, *Mugil cephalus* and *Dorosoma cepedianum* specimens ($n=2$) were obtained from the University of Michigan Museum of Zoology and an *Acipenser brevirostrum* specimen ($n=1$) from the Burke Museum Ichthyological collection (Table 1). Specimens for the gizzard presence survey were either collected via otter trawl or beach seine from locations in the Salish Sea off the coast of Friday Harbor Laboratories or obtained on loan from natural history collections (Table 1). The survey sample consists of both microphagous and non-microphagous fishes and includes specimens spanning 51 species from 45 families. We also surveyed the literature for records of gizzard presence, edentulism (complete or partial, as well as tooth reduction), and microphagy in various fishes and confirmed these reports with dissections when possible. We coded fishes as edentulous if they lack teeth (or robust teeth) in their oral jaws, rather than if species are entirely edentulous, meaning they lack teeth anywhere in the cranial skeleton. This classification of edentulism is more comparable to that of

TABLE 1 The family and species of all fishes included in the taxonomic sample, including the source (museum with catalog number where applicable, or method of collection from Friday Harbor). Collections that provided specimens include the North Carolina Museum of Natural Sciences (NCSM), Burke Museum (UW), California Academy of Sciences (CAS), the University of Louisville (UL), the University of Michigan Museum of Zoology (UMMZ), and Texas A&M University Biodiversity Research and Teaching Collections. The presence or absence of gizzard is noted in the final column.

Family	Species	Source	Gizzard presence
Acanthuridae	*Acanthurus bahianus*	North Carolina Museum of Natural Sciences- NCSM 98958	Yes
Acipenseridae	*Acipenser brevirostrum*	University of Washington- UW 156410	Yes
Agonidae	*Bathyogonus pentacanthus*	Friday Harbor-Otter trawl	No
Agonidae	*Podothecus accipenserinis*	Friday Harbor-Otter trawl	No
Ambassidae	*Ambassis buruensis*	California Academy of Sciences- SU 38113	No
Ammodytidae	*Ammodytes hexapterus*	Friday Harbor- Beach seine	No
Aphredoderidae	*Aphredoderus sayanus*	California Academy of Sciences- ICH 227919	No
Atherinopsidae	*Labidesthes sicculus*	University of Louisville- UL 7550	No
Blennidae	*Salarias fasciatus*	California Academy of Sciences ICH 93577	No
Catostomidae	*Carpiodes* sp. (cf. *cyprinus)*	North Carolina Museum of Natural Sciences- NCSM 3950	No
Centrarchidae	*Pomoxis nigromaculatus*	California Academy of Sciences ICH 27006	Yes
Centropomidae	*Centropomus undecimalis*	Friday Harbor Collection	No
Cichlidae	*Cichlasoma bimaculatum*	Friday Harbor Collection	No
Clupeidae	*Dorosoma cepedianum*	University of Michigan Museum of Zoology UMMZ 156825	Yes
Clupeidae	*Alosa chrysochloris*	University of Louisville- UL 1746	No
Cottidae	*Artedius lateralis*	Friday Harbor- Tide pooling	No
Curimatidae	*Curimatella australis*	University of Michigan Museum of Zoology- UMMZ 207783	Yes
Curimatidae	*Steindachnerina elegans*	University of Michigan Museum of Zoology 216,313	Yes
Cyprinidae	*Ctenopharyngodon idellus*	California Academy of Sciences-SU 29455	No
Cyprinidae	*Campostoma anamolum*	University of Louisville- UL 12224	No
Cyprinodontidae	*Cyprinodon variegatus*	Friday Harbor Collection	No
Elopidae	*Elops saurus*	California Academy of Sciences- SU 51600	No
Embiotocidae	*Cymatogaster aggregata*	Friday Harbor- Beach seine	No
Esocidae	*Esox americanus vermiculatus*	University of Louisville- UL 5605	No
Gadidae	*Theragra chalcogramma*	Friday Harbor- Otter trawl	No
Gerreidae	*Eucinostomus gula*	California Academy of Sciences-SU 36329	No
Gobiesocidae	*Gobiesox maendricus*	Friday Harbor- Tide pooling	No
Haemulidae	*Haemulon sciurus*	California Academy of Sciences-SU 337	No
Hexagrammidae	*Hexagrammus stelleri*	Friday Harbor- otter trawl	No
Hiodontidae	*Hiodon alosoides*	California Academy of Sciences-ICH 244649	No
Ictaluridae	*Ictalurus punctatus*	University of Louisville- UL 8966	No
Liparidae	*Liparis cyclopus*	Friday Harbor- Tide pooling	No
Lutjanidae	*Lutjanus apodus*	California Academy of Sciences-ICH 233145	No
Mugilidae	*Mugil cephalus*	University of Michigan Museum of Zoology- UMMZ 163610	Yes
Mullidae	*Parupeneus barberinus*	California Academy of Sciences ICH 59098	No
Paralichthyidae	*Citharichthys sordidus*	Friday Harbor- Otter trawl	No
Percidae	*Perca flavescens*	University of Louisville- UL 50	No
Pholidae	*Apodichthys flavidus*	Friday Harbor- otter trawl	No
Pholidae	*Pholis laeta*	Friday Harbor- Beach seine	No
Pleuronectidae	*Microstomus pacificus*	Friday Harbor-Otter trawl	No
Pleuronectidae	*Parophrys vetulus*	Friday Harbor-Otter trawl	No
Polypteridae	*Polypterus palmas*	California Academy of Sciences- SU 66380	No

(Continues)

TABLE 1 (Continued)

Family	Species	Source	Gizzard presence
Prochilodontidae	*Prochilodus affinis*	University of Michigan Museum of Zoology	No
Psychrolutidae	*Myoxocephalus polyacanthocephalus*	Friday Harbor-Otter trawl	No
Salmonidae	*Oncorhynchus mykiss*	Friday Harbor-Otter trawl	No
Scombridae	*Scomber scombrus*	California Academy of Sciences SU 13633	No
Siganidae	*Siganus spinus*	California Academy of Sciences SU 32327	No
Stichaeidae	*Xiphister mucosus*	Friday Harbor- Beach seine	No
Zoarcidae	*Lycodes pacificus*	Friday Harbor-Otter trawl	No

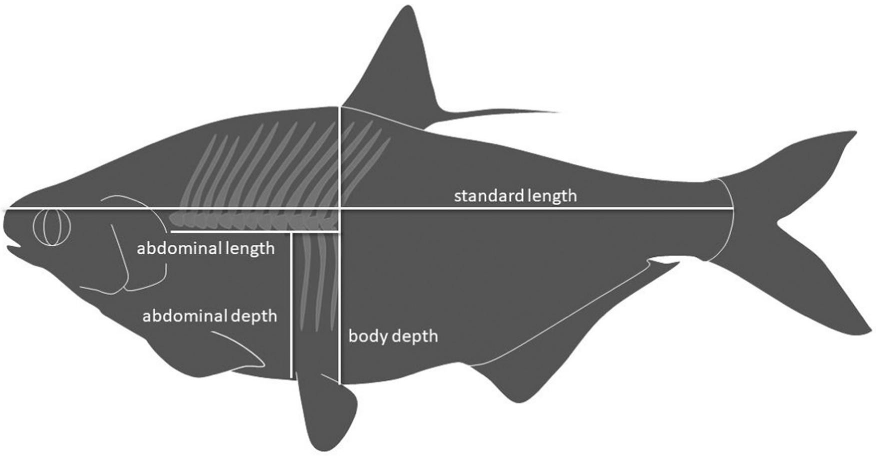

FIGURE 1 A schematic of a fish indicating the basic morphometric characters that were collected. These include standard length (tip of nose to caudal peduncle), abdominal length (tip of nose to anus), body depth (depth at widest point), and abdominal depth (the widest point between the vertebral column and ventral surface of the body). All measurements were recorded in millimeters.

vertebrates in general, which are classified as edentulous if they lack teeth in their oral jaws only.

Fishes without a robust oral dentition may instead use their pharyngeal jaws for processing prey. To explore whether the presence of a robust pharyngeal dentition negates the need for a gizzard, we first coded fishes in our dataset as having robust pharyngeal jaws or not. We coded fishes as having a robust pharyngeal jaw system, according to whether they have been classified as 'true' pharyngognaths (Wainwright et al., 2012, and sources therein) or if species had at least one morphological aspect in common with pharyngognaths (e.g., fused or sutured ceratobranchials, a muscular sling, or a diarthrosis or other robust interaction of the upper pharyngeal jaws with the basicranium; Stiassny & Jensen, 1987; Wainwright et al., 2012). For this latter scenario, we also included cyprinids, catostomids, and leuciscids, which, while not pharyngognaths or even adjacent morphotypes, do have particularly robust pharyngeal jaws and dentitions (Eastman, 1977; Gidmark et al., 2015; Hernandez & Cohen, 2019; Pos et al., 2019).

Prior to dissection, specimens were photographed with a scale bar and ID tag using an iPhone X camera. Basic morphometrics, such as body length and depth, were measured from these images (Figure 1) using FIJI-ImageJ (Schneider et al., 2012). For dissection, we used a razor blade to make a medial incision at the base of the branchial basket, following the midline posteriorly and terminating at the anus. Then, we made an incision just ventral to the vertebral column, following the operculum, such that the entire abdominal cavity was exposed. Specimens were photographed again to create a record of the intact gastrointestinal tract and to collect the morphological characters of abdominal length and depth. The gastrointestinal tract was cut at the anterior margin of the esophagus and the posterior margin of the hindgut and removed from the body cavity. Upon removal, gizzards were excised if present, bisected longitudinally, and then measured for length and width. Lengths were also collected for the foregut (tract prior to the gizzard) and hindgut (tract after the gizzard). In some cases, the hindguts were carefully unwound so their length could be measured. We chose not to measure gut morphometrics from our specimen of *Polyodon spathula* to avoid damaging a large, rare specimen. Similarly, our record for gizzard presence in *Pomacanthus sexstriatus* was taken from Konow and Bellwood (2011). These two species, *Polyodon* and *Pomacanthus*, were thus excluded from morphometric analyses, leaving a total of 49 species.

2.2 | Histology

All gizzards ($n = 3$, 1 per species) were stored in 70% ethanol (EtOH) following dissection, and then prepped for paraffin histology, beginning with a stepwise dehydration series (stored at 70%, 30 min at 90%, 15 min at 100% EtOH). Once dehydrated, samples were placed in 100% xylene for about 24 h until translucent; they were then placed in a 50/50 xylene/paraffin solution for 1 h, followed by three rounds in fresh 100% paraffin for 90 min. Following infiltration, the longitudinal halves of the gizzards were embedded in paraffin and sectioned using a metal blade on a Leica HistoCore BIOCUT Rotary Microtome (Leica Biosystems Inc.) at 5.0 micrometers (μm). After slicing, sections were mounted on glass microscope slides and stained using Mayer's Hematoxylin and Eosin Y (Leica Biosystems Inc.), then imaged using a light microscope affixed with a fiberoptic Nikon camera. Images were processed in Photoshop (Adobe Inc., 2019). Image processing included increasing the resolution by adjusting the pixel size, white balancing, color balancing, and cropping.

2.3 | Phylomorphospaces, ancestral state reconstructions, and evolutionary correlations

Morphometric data were size-corrected against standard length (mm; tip of the snout to the caudal peduncle) by regressing standard length against each morphometric measure. Since size evolution is influenced by phylogeny, we used the '*phyl.resid*' function in *phytools* (Revell, 2009) to extract the size-corrected morphometric residuals while accounting for phylogeny. These morphometric data (not including standard length) were then input into a phylogenetic principal components analysis (PCA) using the '*phyl.pca*' function in *phytools*. The arrangement of these taxa in morphospace was visualized with the '*phylomorphospace*' function (Revell, 2012).

We inferred the number of times gizzards evolved with stochastic character mapping using the '*make.simmap*' function in the *phytools* package (Huelsenbeck et al., 2003; Revell, 2012). For this analysis, we used a time-calibrated phylogeny including all ray-finned fishes and trimmed it to include only the fishes in our sample using the '*drop. tip*' function in the *fishtree* package (Chang et al., 2019; Rabosky et al., 2018). We compared the fit of three models of discrete trait evolution using the corrected Akaike information criterion (AICc): equal rates (ER), symmetrical (SYM), and all rates different (ARD) and chose the model with the lowest AICc score. We then mapped gizzard presence onto the trimmed phylogeny with 1000 SimMap iterations. We fixed the node state for the most recent common ancestor (MRCA) of all ray-finned fishes to not have a gizzard by setting $pi = 0$ in the '*make.simmap*' parameters. This is presumably the most likely scenario given the complete lack of gizzards in non-tetrapod sarcopterygians and chondrichthyans. We also ran this analysis again without making any assumptions about the state of gizzards in the ray-finned fish MRCA.

Similarly, we also performed ancestral state reconstructions for edentulism, pharyngognathy, robust pharyngeal jaws, and microphagy across the phylogeny with stochastic character maps. We classified fishes as microphagous if they ingested some combination of algae, detritus, and phytoplankton. We used generalized linear mixed models (GLMM) for binary data to test for a correlation between gizzard presence and edentulism or microphagy. Fishes stand out among vertebrates in that many clades frequently have two sets of jaws (oral or pharyngeal). Lacking teeth in the oral jaws is considered edentulism in all other vertebrate lineages, so we grouped all fishes that have severely reduced or lost oral dentition as 'edentulous' for our purposes. We performed these analyses in a phylogenetic context using the binary PGLMM function in the ape package (Paradis et al., 2004). This method performs linear regression on binary data, with the phylogeny converted to a variance–covariance matrix, and uses both penalized likelihood and restricted maximum likelihood to estimate the mean and variance of the data, respectively.

A principal component analysis was used to assess the correlation between gizzard presence and morphological traits including foregut length, hindgut length, abdominal depth, body depth, the presence of oral teeth or pharyngeal teeth, and microphagy. We also examined the relationship between gizzard presence and diet. We performed a literature review to understand what prey items are found in the stomachs of fishes with and without gizzards. We considered fishes consuming any combination of detritus, algae, plankton, or plant matter as microphagous (Table S1).

3 | RESULTS

3.1 | Phylogenetic survey of gizzard presence

Using dissection, we noted the presence of a gizzard in *Steindachnerina elegans*, *Curimatella australis*, *Dorosoma cepedianum*, *Mugil cephalus*, *Acipenser brevirostrum*, *Pomacanthus sextriatus*, and *Acanthurus bahianus*. Fishes were considered to have a gizzard if there was grit present in a muscular stomach, although the degree of muscularity differed among species. We were not able to confirm the presence of a gizzard in the African arowana, *Heterotis niloticus*, and the assertion by Horn et al. (2011) that a gizzard is present in this species is not supported by a reference. We also could not confirm the presence of a gizzard in any *Carpiodes* species (Catostomidae), *contra* Brezner (1958), or in *Polyodon* (see Weisel, 1973). However, we did find evidence of a gizzard in *Ictiobus* (not included in analyses), which looks similar to *Carpiodes* and could have been misidentified in Brezner (1958). The majority of sampled families did not have a gizzard (Table 1).

3.2 | Gizzard morphology and cellular histology

The gizzards of *D. cepedianum*, *M. cephalus*, and *A. brevirostrum* varied widely in their gross anatomy, cellular composition, and cellular organization. In all fishes with gizzards from our sample, the gizzard is located between the esophagus and hindgut; these fishes also lacked a stomach. The gizzards of all species were filled with sand or some other mineral particle (i.e., 'grit'). In the species where a size range of individuals were dissected (*S. elegans*, *C. australis*, *D. cepedianum*, *and M. cephalus*), gizzard mass and length increased with standard body length.

The gizzard of *D. cepedianum* is walnut-shaped with a uniform distribution of a high volume of smooth muscle. It is the most muscular of all sampled gizzards, with thick pads of circumferential smooth muscle down the length of the gizzard (Figure 2a, white arrowhead; Figure 2b). It also has the least diverse cellular composition of the gizzards; the muscular layer is connected to glandular cells (Figure 2a, black arrowhead; 2c) via a thin layer of dense, regular connective tissue (Figure 2a, white arrow). Internal to the glandular tissue and interfacing with the lumen is ciliated epithelium (Figure 2a, black arrow).

Gizzards found in *M. cephalus* were more robust than in other species, with their anterior portion being characterized by hypertrophied smooth muscle; this gave *Mugil* gizzards an acorn shape not found in other fish gizzards. There are thick pads of smooth muscle composed of both circumferential (Figure 3a, white asterisk) and longitudinal fibers (Figure 3a, white arrowhead; 3d, white arrowhead). *M. cephalus* has the most histologically complex gizzard; there are mucosal folds near the esophageal margin that interface with the lumen (Figure 3a, black asterisk; 3b). There are glandular cells that interface with the lumen toward the posterior margin (Figure 3a, black arrowhead; 3c). There is a thick pad of adipose tissue between pads of smooth muscle (Figure 3a, black arrow) and a robust blood vessel along the length of the gizzard (Figure 3d, black arrow).

The gizzard of *A. brevirostrum* has a unique integration with the esophagus, where the gizzards of *D. cepedianum* and *M. cephalus* were composed mostly of muscle near the esophageal margin, the sturgeon gizzard is interspersed with spongy connective tissue (Figure 4a, black arrow; 4b). The smooth muscle is composed of both circumferential and longitudinal fibers, as observed in *M. cephalus* (Figure 4a, white arrowhead; 4c). Toward the lumen, there is a mucosal layer that is composed of smooth muscle interspersed with glandular tissue (Figure 4a, white arrowhead; 4c). Goblet cells are indicated with a black arrow in Figure 4c.

3.3 | Evolutionary reconstructions

Based on AICc scores, the all rates different (ARD) model was supported as the best fit to the gizzard presence/absence data

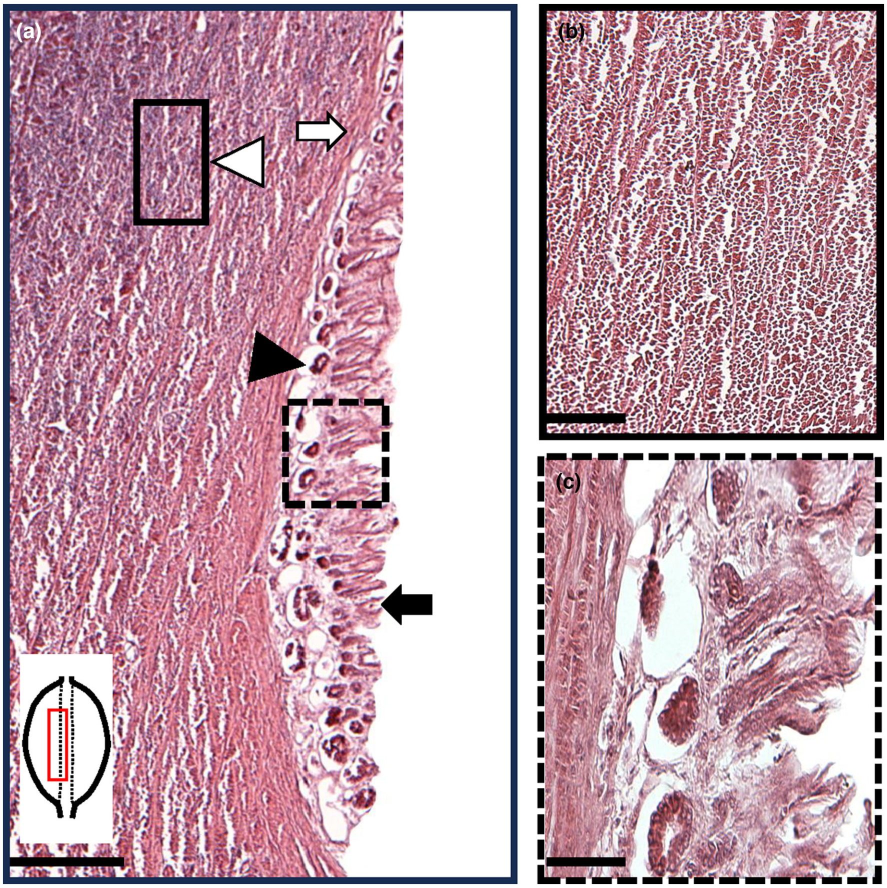

FIGURE 2 A longitudinal section of the gizzard of *Dorosoma cepedianum* (standard length 72.14 mm) stained with Meyer's Hematoxylin and Eosin. The schematic of a bisected gizzard in the lower left corner illustrates the region from which tissue was sampled. (a) Is an overview of gizzard cellular morphology. The white arrow indicates dense, regular connective tissue, and the white arrowhead indicates circumferentially oriented smooth muscle. The black arrowhead indicates glandular tissue, and the black arrow indicates cilia that interface with the lumen. (b) Showcases circumferentially oriented smooth muscle; (c) Indicates glandular tissue and cilia. Scale set to 200 μm (a) and 50 μm (b,c).

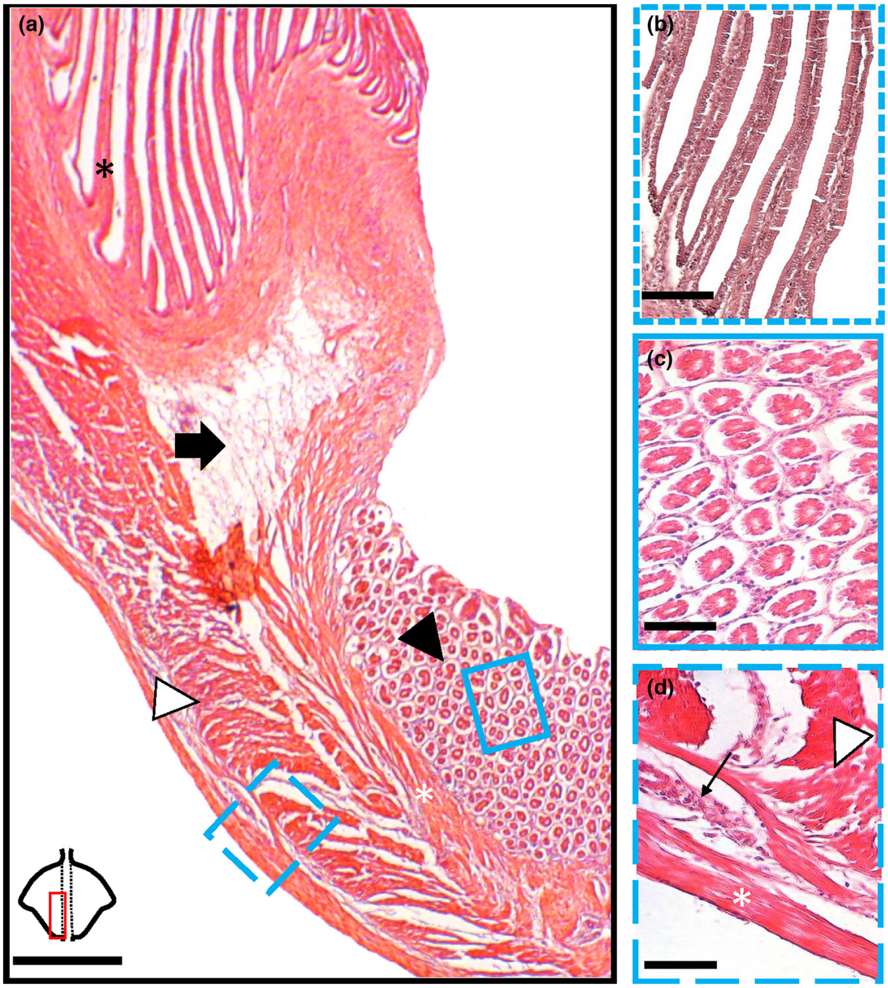

FIGURE 3 A longitudinal section of the gizzard of *Mugil cephalus* (standard length 118.41 mm) stained with Meyer's Hematoxylin and Eosin. The schematic of a bisected gizzard illustrates the region of sampling. (a) Is an overview of gizzard cellular morphology. The white arrowhead indicates circumferential smooth muscle; the white asterisk indicates longitudinal smooth muscle. The black asterisk illustrates the mucosal folds, shown at higher magnification in (b). The black arrowhead indicates glandular tissue, which interfaces with the lumen and is magnified in (c). The black arrow points to adipose tissue. (d) Showcases the longitudinally oriented (white asterisk) and circumferentially oriented (white arrowhead) smooth muscle fibers, separated by a large blood vessel. The scale is set to 200 μm (a) and 50 μm (b–d).

(−lnL: −18.78, AICc: 42.82; ER & SYM AICc = 42.2). According to our sample, gizzards have evolved at least six times across ray-finned fishes and are only sporadically present across clades. Gizzards were 1.3× more likely to evolve than be lost, according to a scenario where the common ancestor of all ray-finned fishes lacks a gizzard. In a scenario where the MRCA node state was allowed to vary, stochastic character maps estimated a 50/50 chance of this hypothetical ancestor having a gizzard. Likewise, this analysis reconstructed a near equal chance for gizzards to be lost vs. gained (10.006 vs. 11.546, respectively). Gizzards were inferred to evolve infrequently, with lineages spending 88% of their evolutionary history without gizzards, according to stochastic character map data. We documented that gizzards are almost always associated with edentulism over microphagy (Figure 5; Table S1). Additionally, generalized linear mixed model results show a significant relationship between edentulism and gizzard presence ($p = 0.016$), but only a near-significant relationship between gizzards and microphagy ($p = 0.058$). However, we did observe that gizzards and microphagy often co-occurred: 1 of 8 species with a gizzard was not microphagous, while 14 of 21 microphagous species did not have gizzards. There was no relationship of gizzards to either pharyngognathy ($p = 0.367$) or lineages having robust pharyngeal jaws in general ($p = 0.071$).

Gizzards are present in almost all the major actinopterygian lineages: teleosts and non-teleosts (*Acipenser*), clupeocephalans and otocephalans (e.g., *Dorosoma*, *Steindachnerina*), and acanthopterygians, including percomorphs (e.g., *Pomacanthus*, *Acanthurus*). This study also documents the prevalence of gizzards in the characiform lineage Curimatoidea (e.g., Curimatidae and Prochilodontidae; see Fugi et al., 2001; Menezes & Caramaschi, 2007). The gizzard in curimatids may be widespread among different genera and thus is a candidate synapomorphy for the family. The earliest branching teleost lineage to have a gizzard is the Otocephala, which contains several species with gizzards, such as *Dorosoma* spp.

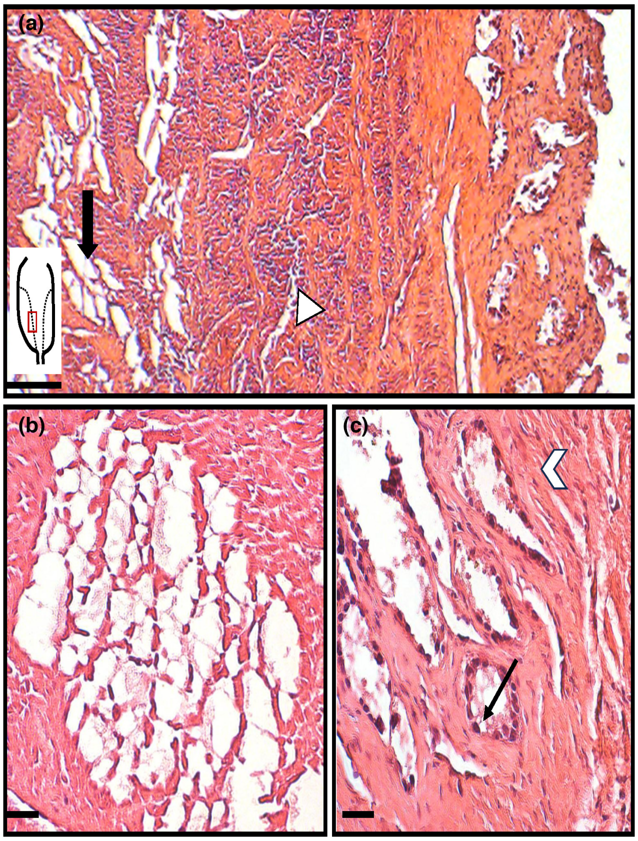

FIGURE 4 (a) An overview of the cellular morphology of the gizzard of *Acipenser brevirostrum* (standard length: 178.54 mm), showing the outer muscular layer and inner mucosa. The schematic of a bisected gizzard illustrates the region of sampling. The black arrow indicates the spongy tissue found throughout the gizzard, which is magnified in (b). (c) Illustrates the longitudinal smooth muscle (white arrow) and glandular tissue, with a goblet cell pointed to by the black arrow. The scale is set to 200 μm (a) and 50 μm (b, c).

3.4 | Phylomorphospace

According to the broken stick analysis, only the first two PC axes were informative. PC1 accounted for 39.9% of the total variance, while PC2 accounted for 22.9%. All morphometric variables loaded negatively on PC1. Hindgut length, body, and abdominal depth loaded positively on PC2, while foregut and abdominal length loaded negatively (Table 2). Lineages with gizzards occupied a smaller total region of morphospace than lineages without gizzards (Figure 6). Specifically, fishes with gizzards were generally characterized by near-mean values along PC1 and positive values of PC2. This suggests that fishes with gizzards generally have longer hindguts and deeper bodies, as is characteristic of herbivorous or detritivorous fishes (Burns, 2021). *Carpiodes cyprinus* (Catostomidae) was representative of gut and body dimensions at the negative and positive extremes of PC1 and PC2, respectively, with a deep body and abdomen and a long hindgut. Similarly, the English sole (Pleuronectidae; *Parophrys vetulus*) loaded negatively on PC1 and PC2, with a long, deep body and a long foregut. *Xiphister mucosus*, an eel-like prickleback (Stichaeidae), ordinated to the extreme positive end of PC1 and was characterized by a long body, a shallow abdomen, and a short gut (Figure 6).

4 | DISCUSSION

4.1 | The nitty-gritty details of gizzard functional evolution

We find that gizzards have evolved at least six times across ray-finned fishes, compared with only once or twice in extant archosaurs. Thus, gizzards have evolved more frequently in fishes than any other living vertebrate lineage. The infrequent evolution of gizzards begs discussion of why these structures are so rare in fishes and in vertebrates in general (Fritz et al., 2011; Wings & Sander, 2007). There are several candidate hypotheses that could explain the evolution and functional significance of gizzards. Fritz et al. (2011) and others suggest that gizzards are a substitution for teeth in edentulous animals and represent a consequence of evolutionary contingency and constraint. We also propose another hypothesis, based on Lucas' (2004) ruminations on the mechanics of tooth function and food size. Lucas' model dictates that, at some minimum threshold, a large triturating surface (like a tooth) cannot induce crack propagation in diminutive food items. Instead, exceedingly small food particles deform when compressed between larger surfaces (Lucas, 2004). We extend Lucas' logic to gizzards and propose that gizzards, along with ingested gastroliths, are fundamentally adapted for triturating extremely small food particles. We propose that the small size of grit (relative to teeth) and the even smaller shearing faces of grit particles may mitigate Lucas' minimum threshold. Finally, it is possible that using a gizzard to process food is a means to maximize the animal's metabolic investment in the gut (Van der Schoor et al., 2002) and avoid investment in teeth, which are prone to declining performance over time due to wear and breakage (Veiberg et al., 2007). Thus, gizzards may be a cost-effective mechanism that still facilitates the breakdown of tough food (Louchart & Viriot, 2011). We will refer to these ideas as the (1) *contingency-constraint*, (2) *minimum-threshold*, and (3) *metabolic-savings* hypotheses, respectively.

The contingency-constraint hypothesis suggests that edentulous fishes modify their existing gut to process intractable foods. Our data support this hypothesis, demonstrating that tooth reduction or loss often corresponds with gizzard presence (Figure 6). These observations invoke a ‘chicken or the egg’ dilemma: do gizzards evolve as a response to edentulism, or do organisms abandon teeth because they have a gizzard? Previous research shows that phytoplanktivoy and detritivory exclusively evolved from zooplanktivory in clupeiforms (Egan et al., 2018). Furthermore, zooplanktivory is generally associated with small, few teeth in clupeiforms (Grande, 1985; Whitehead, 1985; Whitehead et al., 1988). Taken together, these patterns, along with our documentation of gizzards in all of the microphagous clupeiforms we examined for this study, suggest that reductions in dentition preceded gizzard evolution in

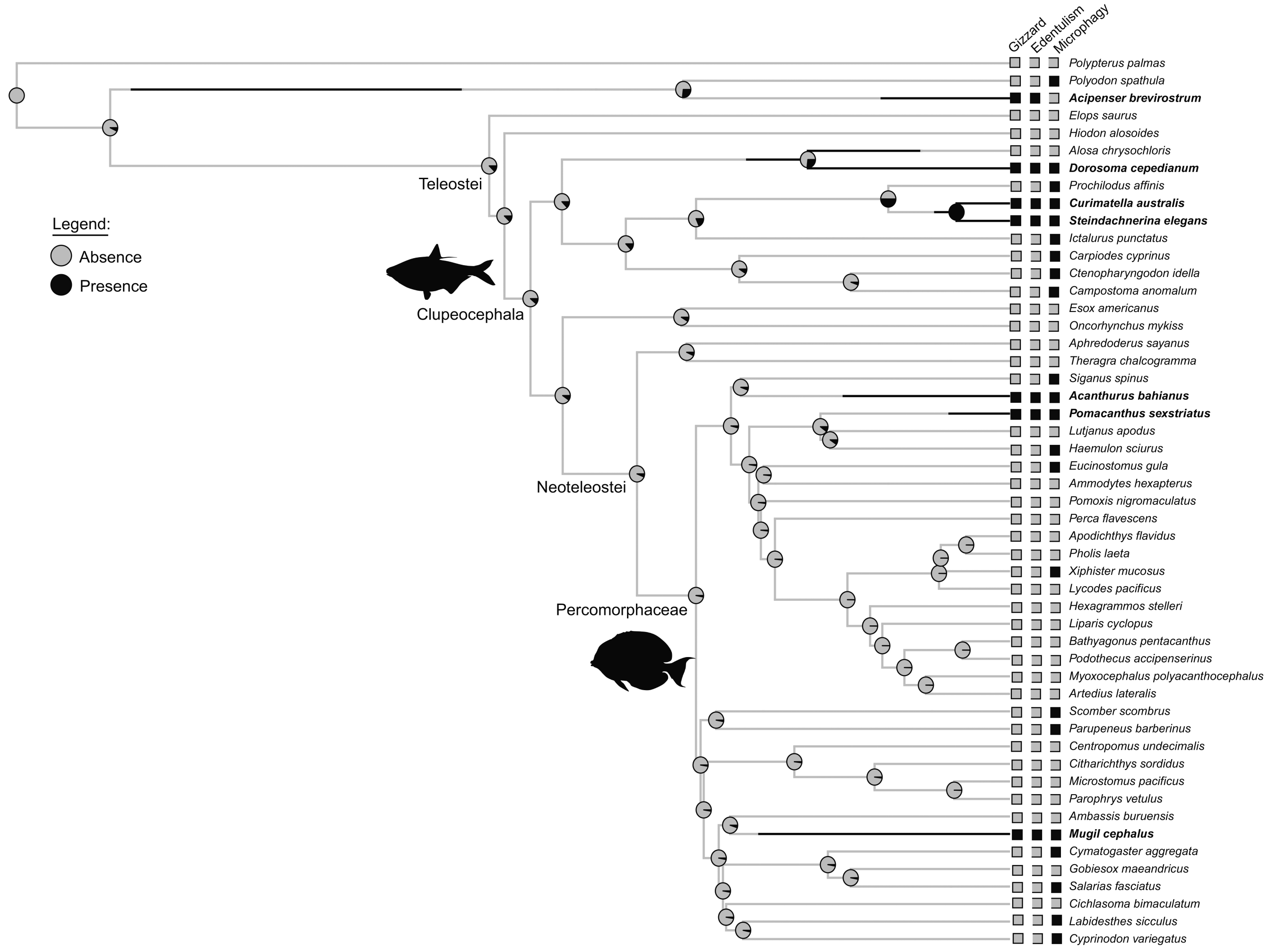

FIGURE 5 The reconstruction of the evolution of gizzard presence, edentulism, and microphagy in our sample of bony fishes using stochastic character mapping with the most recent common ancestor (MRCA) fixed to not having a gizzard. The fish icon at Clupeocephala represents *Dorosoma*, and the fish icon at Percomorphaceae represents *Acanthurus*. This reconstruction is based on the phylogeny by Rabosky et al. (2018).

TABLE 2 Values for the principal component analysis (PCA) underlying ordination for the phylomorphospace in Figure 6.

Character (mm)	PC1	PC2	PC3	PC4	PC5
Hindgut length	−0.7595411	0.50242383	0.3452016	0.07858929	−0.2129017
Foregut length	−0.7015304	−0.63260359	0.1055898	0.29382889	−0.1009120
Body depth	−0.8223446	−0.01820131	−0.4898853	−0.22548780	0.1805150
Abdominal length	−0.8407299	−0.18728833	0.3001519	−0.36846446	0.1795523
Abdominal depth	−0.8569661	0.27376312	−0.2167663	0.26767233	0.2683781

clupeiforms. In extinct archosaurs, the situation is more complex, with taxa sometimes having both gizzards and teeth, but tooth loss still typically precedes gizzard evolution (Louchart & Viriot, 2011; Zheng et al., 2011). Other anatomical considerations might also reinforce the relationship between gizzards and tooth reduction; the evolution of a gizzard in birds may also have been a method to shift mass away from the head and towards the center of gravity, improving the efficiency of flight (Dilger, 1957; Fritz et al., 2011). Given the evolutionary association between edentulism and gizzards, we assert that gizzards represent a morphological and functional innovation that arose in response to edentulism.

Interestingly, the presence of a gizzard was not correlated with the presence of pharyngeal jaws. We suggest three possibilities: (1) that the mechanical processing performed by pharyngeal jaws and gizzards is not synonymous; (2) that the gizzard may be a suitable substitute for an oral dentition but not for pharyngeal jaws; and

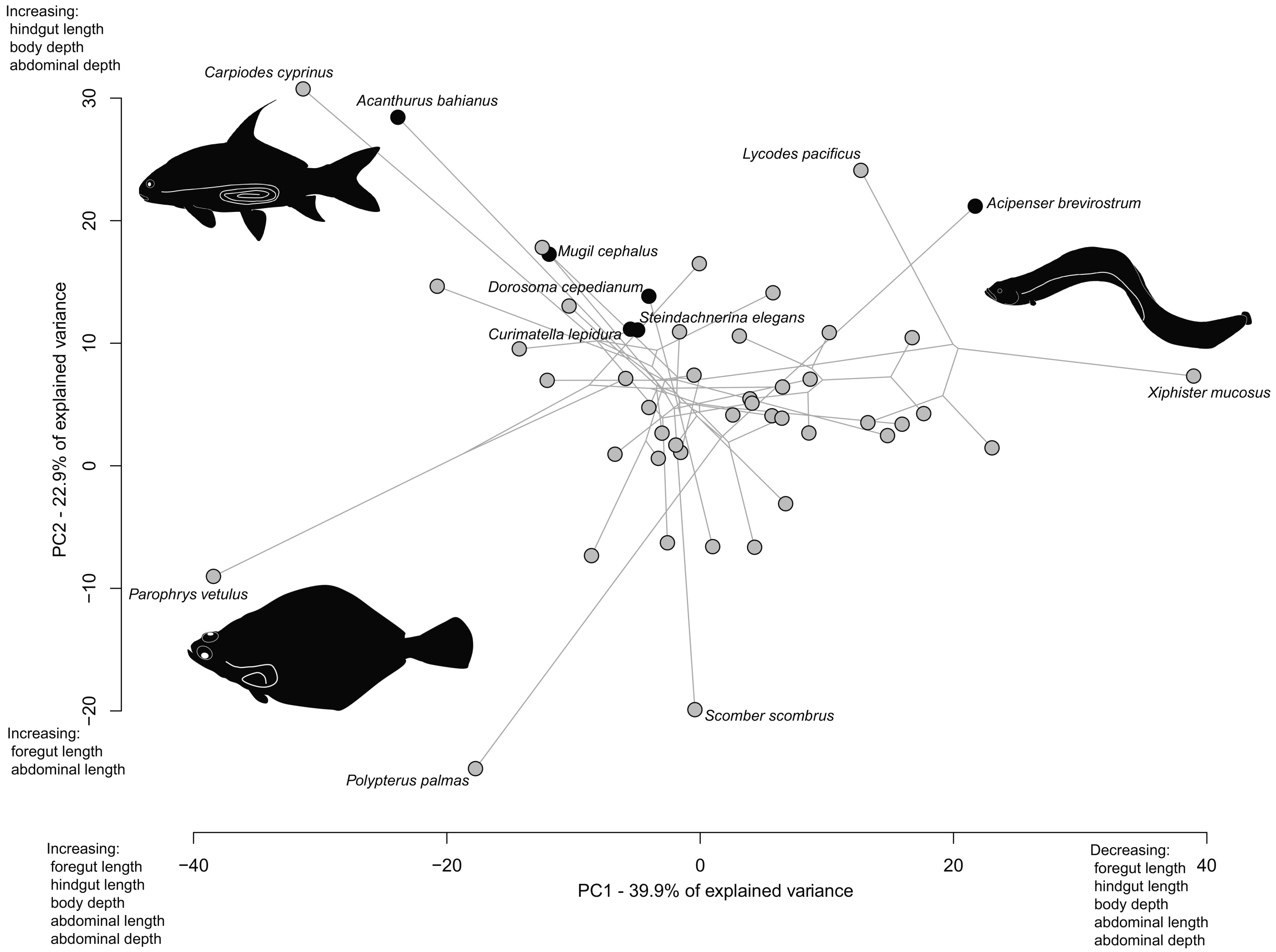

FIGURE 6 A phylomorphospace of the first two principal components of gizzard presence and body morphometrics across bony fishes. A filled circle (tip) represents each species included in our sample; gray circles are species without gizzards, and black circles are species with gizzards.

(3) that oral edentulism can vary independently of pharyngeal dentitions in fishes. It may be that multiple mechanisms of prey reduction are still necessary in many fishes. More study is needed regarding the form and function of pharyngeal jaws in fishes, beyond the narrowly defined 'pharyngognaths' and their allies, to resolve the roles of pharyngeal jaws in prey breakdown.

Although we did not find statistical support for the minimum threshold hypothesis, we did find that microphagy does frequently coincide with gizzards in fishes and that nearly all gizzard-bearing fish are microphagous. Gizzards may still be circumventing the minimum size of prey that can be reduced by teeth by performing an additional role that dentitions cannot. We contend that the gizzard serves two simultaneous functions: particle aggregation and trituration. Aggregation is accomplished when food particles are trapped in the narrow lumen of the gizzard and bound by mucus, forming a kind of secondary bolus akin to particle aggregation by the epibranchial organ of otomorphan fishes (Cohen et al., 2018, 2022). According to Pos et al. Pos et al. (2021), aggregation and size reduction of food in gizzards are not successful without mucus. Trapped food particles are then triturated when gastroliths in the lumen are forced together by smooth muscle contractions (Figure 2) (Schmitz & Baker, 1969). Given these combined functions and our observation that microphagy frequently co-occurs with gizzards, we posit that a gizzard is a way to "chew" without teeth and serves as a way to circumvent the limits on minimum prey size that teeth impose. While gizzards might be exceedingly useful for processing particularly small food particles, they may be disadvantageous for reducing large prey (Fritz et al., 2011; van Gils et al., 2003). This is especially true in edentulous animals, which have few options for reducing ingested food prior to the gizzard. One reason for the rarity of gizzards is that these organs are less ecologically versatile than teeth. We hypothesize that gizzards operate best over a narrower range of food sizes than comparable dentitions.

Our final proposed explanation for gizzard evolution, the metabolic-savings hypothesis, may be the most difficult to address experimentally and the hypothesis our data are least apt to address. However, the principal assumption of this hypothesis is that both teeth and the digestive tract are metabolically expensive organs (Jonasson et al., 2018; Smith et al., 2017; Van der Schoor et al., 2002). Gizzards have the advantage of capitalizing on existing investment in the gut as well as resisting the wear and decline in performance often experienced by teeth (Carr et al., 2006; Veiberg et al., 2007). Thus, gizzards may be a more cost-efficient way to process food, rather than maintaining both a gut and a dentition. Data in support of the metabolic expense of teeth are currently lacking (Mongle et al., 2020) but they are also central to larger questions in understanding the diversity of tooth replacement modes across vertebrates (Carr et al., 2021; Cohen & Summers, 2023). While data defining the metabolic investment for maintaining a dentition are scarce, current studies in primates suggest that the mineralization of teeth is particularly costly (Smith et al., 2017). However, cellular maintenance of the gut has been experimentally shown to be metabolically intensive (Holmberg et al., 2002; Secor & Nagy, 1994). For example, snakes regularly remodel their gut between feeding bouts, a phenomenon thought to save the cost of maintaining a metabolically expensive organ during periods of disuse (Holmberg et al., 2002; Secor & Nagy, 1994). New approaches and more research are needed to test this hypothesis.

4.2 | Morphological conservation and diversity of vertebrate gizzards

Gizzards, like other components of vertebrate feeding and digestive systems, demonstrate clear functional adaptations related to dietary habits. For example, the hypsodont molars of ungulates resist wear wrought by abrasive endogenous or exogenous plant materials, while the muscular proventriculus of owls aggregates and then ejects pellets of indigestible matter (Raia et al., 2010; Smith & Richmond, 1972). Gizzards in fishes vary in their integration with the rest of the gut tract; specifically, the gizzard found in *Acipenser brevirostrum* (sturgeon) connects to the esophagus via a broad, saclike opening comprised of spongy tissue, not found in other species (Figure 4a). Perhaps the divergent gizzard morphology in sturgeon could relate to the small mollusks these fishes consume. Sturgeon could be using gizzards to crush brittle prey items like some molluscivorous birds (van Gils et al., 2003).

Although the gizzards differ in their cellular composition, they share core tissue types, each with functional implications (Moore, 1998b; Moore et al., 1998). The dominant tissue type found in all fish gizzards is smooth muscle. We hypothesize that as the circumferential smooth muscle of the gizzard contracts, it exerts a force inward toward the lumen, facilitating grinding and mixing (Sanders et al., 2012). When both the circumferential and longitudinal smooth muscle fibers contract, they may generate forces both inward and downward, resulting in translational movement within the gizzard (Moore, 1998b). In addition to generating shear, which fish gizzards certainly share with their avian counterparts, the mucus secreted by goblet cells may help to aggregate high volumes of low-nutrient food (Holley et al., 2015).

In addition to aggregation, nutrient-poor diets often require increased residency time in the hindgut to facilitate nutrient absorption (German & Horn, 2006; Kapoor et al., 1976). An increased residency time is often achieved by lengthening the hindgut and deepening the abdomen (Burns, 2021). Indeed, fishes with gizzards exhibited deeper bodies and longer hindguts, characteristics associated with herbivory and detritivory (Figure 6). This body plan is representative of just a tiny fraction of the possible body morphologies observed across fishes. The relative rarity of gizzards across fishes and the specific morphological characteristics fishes with gizzards share suggest that gizzards are associated with a diet that requires additional effort to digest and absorb nutrients.

5 | CONCLUSIONS

The cellular morphology and gross anatomy of gizzards vary across fishes. Fishes with gizzards are typically deep-bodied and have long hindguts. We found support for our predicted association between microphagy and gizzards, with nearly all fishes with gizzards in our dataset occupying microphagous trophic niches. Thus, despite not finding a statistically significant correlation between gizzards and detritivory, we found qualitative evidence that gizzards play a key role in mechanically processing microscopic food particles in multiple lineages of fishes. We also found evidence that reductions in dentition preceded gizzard evolution in clupeiforms, suggesting that this may be a general pattern in fishes. Given that gizzards evolved in some of the earliest lineages of ray-finned fishes, namely sturgeon (*Acipenser* evolved around 80–150 mya; Brinkman, 1990; Shen et al., 2020), this may suggest that fishes may be some of the earliest vertebrates to evolve gizzards, alongside avian dinosaurs and other archosaurs. Gizzards have also evolved several times independently in fishes, in most major freshwater and marine clades. This research underscores how complex prey processing is widespread across vertebrates and accomplished through diverse means.

AUTHOR CONTRIBUTIONS

All authors contributed to conceiving and designing the study. Sarah D. Arnette and Matthew A. Kolmann performed dissections. Matthew A. Kolmann and Josh P. Egan performed the diet literature review. Sarah D. Arnette collected morphometric data. Sarah D. Arnette, Karly E. Cohen, and Lauren E. Simonitis performed histological sectioning and staining. Sarah D. Arnette and Matthew A. Kolmann analyzed the data. Sarah D. Arnette and Matthew A. Kolmann drafted early versions of the manuscript. All authors contributed to refined versions of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

DATA AVAILABILITY STATEMENT

Histological images are available from the lead author upon request. Morphometric and ecological data can be found in the supplemental appendices.

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