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RESEARCH ARTICLE

Relative size variation of the otoliths, swim bladder, and Weberian apparatus structures in piranhas and pacus (Characiformes: Serrasalmidae) with different ecologies and its implications for the detection of sound stimuli

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Abstract

The Weberian apparatus of otophysan fishes confers acute hearing that is hypothesized to allow these fishes to assess the environment and to find food resources. The otophysan family Serrasalmidae (piranhas and pacus) includes species known to feed on falling fruits and seeds (frugivore/granivores) that splash in rivers, herbivorous species associated with torrents and rapids (rheophiles), and carnivores that feed aggressively within shoals. Relevant sound stimuli may vary among these ecological groups and hearing may be tuned to different cues among species. In this context, we examined size variation of the Weberian ossicles, swim bladder chambers, and otoliths of 20 serrasalmid species from three broad feeding ecologies: frugivore/granivores, rheophiles, and carnivores. We performed 3D-reconstructions of high resolution tomographic data (μCT) from 54 museum specimens to estimate the size of these elements. We then tested for an ecology effect on covariation of auditory structure size and body size and accounted for phylogeny with phylogenetic generalized least squares analyses. Among ecological groups, we observed differences in relative sizes of otoliths associated with sound pressure and particle motion detection, and variation in Weberian ossicle size that may impact sound transmission. Rheophiles, which live in noisy environments, possess the strongest modifications of these structures.

KEYWORDS

asteriscus, intercalarium, lapillus, sagitta, scaphium, tripus

1 | INTRODUCTION

Otophysan fishes are a diverse clade of 77 families and over 10,000 spp. and occur in freshwater habitats worldwide (Nelson, Grande, & Wilson, 2016). The evolutionary success of this taxon has been attributed, in part, to sensitive hearing as a result of an anatomical linkage between the swim bladder organ, the anterior axial skeleton, and the inner ear (Braun & Grande, 2008). This structure, first described by Weber (1820) and now termed the Weberian apparatus, consists of modified vertebrae that form a mechanical linkage between the swim bladder and inner ear (Alexander, 1962; Popper & Fay, 1973). Three pairs of Weberian ossicles (listed caudal to rostral: tripus, intercalarium, and scaphium) connect the anterior swim bladder and the inner ear. A linkage via ligaments connects the ossicles to the sinus impar, a perilymphatic space in the inner ear (Alexander, 1962; Chardon & Vandewalle, 1997; Popper & Fay, 1973). In addition to the ossicles, a small claustrum bone lies dorsal to the scaphium (Braun & Grande, 2008; Chardon & Vandewalle, 1997) but is not connected by these ligaments and thus may not be directly involved in sound vibration transmission (Braun & Grande, 2008). The gas filled swim bladder, because of its impedance mismatch with the surrounding water environment, vibrates from the pressure wave component of sound (Fay & Popper, 1974, 1975; Yan, 2004). Weberian ossicles transduce these vibrations into endolymphatic fluid flow that stimulates the saccules of the inner ear (Chardon & Vandewalle, 1997; Finneran & Hastings, 2000). The primary pathway of sound detection in fishes involves direct stimulation of the otolithic organs by whole-body accelerations that are induced by the particle motion component of sound (Popper & Fay, 2011; Popper, Fay, Platt, & Sand, 2003). Because fish tissues are of a similar density to water, the pressure component of sound passes through the body without inducing accelerations, but the swim bladder and Weberian apparatus provide indirect sound pressure stimulation of the ears (Fay & Popper, 1974, 1975; Yan, 2004).

In addition to modifications of the anterior vertebrae, otophysan fishes have modifications in otolith size and shape relative to other teleost fishes (Fay, 1984; Popper & Fay, 2011). While there is considerable variation in otolith size and shape among teleosts (Cruz & Lombarte, 2004; Lychakov & Rebane, 2000; Popper, Ramcharitar, & Campana, 2005), in most teleost species, the sagitta (saccular otolith) tends to be the largest otolith, shows the greatest variation among species in shape, and is hypothesized to have the strongest association to hearing (Ladich & Schulz-Mirbach, 2016; Popper & Fay, 2011). Otophysans differ from most teleosts in having asterisci (lagenar otoliths) that are larger than the sagittae, have elaborate shapes, and are hypothesized to be more involved in detection of particle motion, while the saggitae are small and elongate and are hypothesized to be involved with indirect detection of sound pressure stimuli transduced by the Weberian apparatus (Fay, 1984; Popper & Fay, 2011). The small size of otophysan sagittae may reduce their inertia and is consistent with their function of stimulation from endolymphatic flow transduced by Weberian ossicle motion rather than whole-body acceleration (Chardon, Parmentier, & Vandewalle, 2003). The utriculus is thought to function primarily in the vestibular sense in most fishes (Ladich & Schulz-Mirbach, 2016). In otophysans, the lapillus (utricular otolith) is relatively large (Assis, 2005) and the utriculus may have an additional auditory role in detection of sound particle motion (along with the lagena; Fay, 1984; Popper & Tavolga, 1981). It is hypothesized that all three otolithic endorgans may have some dual function in hearing and the vestibular sense, but vary in their primary functions (Popper et al., 2003).

Sensitive hearing in otophysans has long been appreciated (von Frisch, 1938) and studies of otophsan fishes often show hearing ranges extending to 3 kHz or more (Ladich, 1999; Popper, 1972; Yan, Fine, Horn, & Colón, 2000). This condition is thought to provide increased sensitivity to sound pressure relative to teleost species with an ancestral hearing morphology, though notably, some other teleost lineages have independently evolved accessory hearing morphologies associated with increased sensitivity and bandwidth (e.g., Clupeiformes, Anabantifomes, etc.; Ladich, 2013; Ladich & Schulz-Mirbach, 2016). As frequency increases, sound particle motion attenuates more rapidly with source distance compared to sound pressure and thus fishes with pressure sensitivity are able to detect higher sound frequencies at a greater distance from the source (Popper et al., 2003). This novel morphology is hypothesized to be of importance in the evolutionary success of this group of fishes, yet the main selective pressures and adaptive benefits of acute hearing remain debated (Ladich, 2000). Sound production is known from many catfishes (Siluriformes), but is thought to be far less common among the other three otophysan orders and, in some cases, there is a substantial mismatch between the best frequencies of hearing and the main spectral energy of otophysan vocalizations (Ladich, 1999). Vertebrate hearing may have evolved as an adaptation to allow animals to sense the auditory scene (Fay & Popper, 2000; Popper et al., 2003; Popper & Platt, 1993), that is, information about the types and locations of different sound sources. Such scene analysis could allow fish to accomplish fundamental tasks like detecting predators and prey (Fay & Popper, 2000; Popper et al., 2003). It has been hypothesized that high-frequency hearing in teleosts like otophysans may have evolved in quiet, shallow, freshwater environments that have low background noise and for which the cut-off frequency imposed by the interaction of shallow depths and long wavelengths limits detection of lower frequencies (Ladich & Popper, 2004; Popper et al., 2003; Rogers & Cox, 1988). The hypothesis that enhanced hearing sensitivity driven by selection pressures for auditory scene analysis is intriguing in light of the evolutionary success and trophic specialization of otophysan fishes in freshwater environments. A recent study on a group of North American cyprinid fishes found evidence of the role of hearing for detection of indirect feeding cues, in this case, the sounds of shuffling rocks in streams that may be associated with events that dislodge invertebrate prey (Holt & Johnston, 2011). Thus, additional studies are necessary to examine the role of hearing in the ecology of other otophysan fish families that present a variety of feeding habits and variation of the Weberian anatomy that may affect their hearing sensitivity.

The Weberian apparatus exhibits morphological variation within and among the four otophysan orders of fishes (Alexander, 1962; Bird & Hernandez, 2007; Chardon et al., 2003; Lechner & Ladich, 2008). The functional consequences of this morphological variation for hearing sensitivity, however, have rarely been examined (Lechner & Ladich, 2008). Variation in the Weberian apparatus morphology of catfishes, which includes swim bladder size and shape and Weberian ossicle number and shape, is correlated with hearing range differences among catfish families (Lechner & Ladich, 2008). It remains to be seen if size variation among species within other otophysan orders is associated with hearing ability and how the associated hearing sensitivities may be important in different ecological and behavioral contexts.

The functional morphology of the Weberian apparatus in the order Characiformes remains poorly studied. This otophysan order is speciose (24 families, ~2,300 species), reaches its greatest diversity in South America (Nelson et al., 2016), and includes the family Serrasalmidae. Serrasalmidae is thought to comprise 93 valid species (Eschmeyer & Fong, 2017) and 16 extant genera (Thompson, Betancur, Lopez-Fernandez, & Ortí, 2014) and includes ecologically and commercially important species and the familiar piranhas and pacus (Goulding, 1980). Functional aspects of size variation in auditory structures and its adaptive consequences among serrasalmid fishes remain largely untested. Studies on hearing in serrasalmids confirm the presence of sound pressure sensitivity that extends to relatively high-frequencies (>3 kHz) (Ladich, 1999; Mélotte, Parmentier, Michel, Herrel, & Boyle, 2018; Stabentheiner, 1988). Hearing threshold data from many species within the family, however, are lacking and the relative role of sound pressure and sound particle motion sensitivity is not known for any serrasalmids. In addition, the adaptive significance of hearing sensitivity and its role in ecology and behavior for serrasalmids remains speculative.

Several hypotheses for the evolution of acute hearing in serrasalmid fishes have been put forth. Sound production is known from several piranha species (Ladich, 1999; Markl, 1971; Mélotte, Vigouroux, Michel, & Parmentier, 2016; Millot, Vandewalle, & Parmentier, 2011; Stabentheiner, 1988), however, studies on piranha hearing indicate that the bandwidth of hearing extends to frequencies much higher than the main energy present in their sounds. Thus, other selection pressures related to the mode-of-life of these fishes may favor high frequency hearing. Another hypothesis for the evolution of acute hearing in serrasalmids is prey detection (Ladich, 1999; Mélotte et al., 2018; Stabentheiner, 1988). Splashing noises are reported to be attractive to piranhas (Markl, 1972; Mol, 2006; Stabentheiner, 1988) and such sounds could occur from wounded prey and other piranhas feeding at the water surface. Splashing sounds from fishes at the water surface contain high-frequency components that can extend from one to several kHz (Bolgan, O'Brien, Rountree, & Gammell, 2016; Phillips, 1989). In shallow waters, such as flooded tropical forests, high frequency components of such sounds would be predicted to carry further from the sound source than lower frequency components that may not carry far because of their longer wavelengths (Rogers & Cox, 1988). Further, a variety of herbivorous and omnivorous serrasalmids consume falling fruits and seeds (Horn et al., 2011) and there are numerous accounts that indicate the sound of falling fruits may attract frugivorous serrasalmids (Correa, Winemiller, López-Fernández, & Galetti, 2007; Gottsberger, 1978; Parolin, Waldhoff, & Piedade, 2010; Piedade, Parolin, & Junk, 2006). It is not known, however, how these fishes detect the sounds of these events. The hypothesis that the ability to locate prey is a major selective force in the evolution of hearing in fishes has been suggested in numerous reviews (Ladich, 1999; Myrberg, 1981; Popper et al., 2003; Popper & Platt, 1993; Popper & Schilt, 2008), yet this topic has received very little experimental research attention (Holt & Johnston, 2011). Thus, investigations into the relationship between hearing and the ecology of fishes are expected to shed light on how natural selection has shaped the evolution of the auditory system. The diversity of serrasalmid fishes, the piranhas and their relatives, provides an important opportunity to address this topic.

The goal of this study was to characterize the size variation of otoliths and accessory auditory structures of serrasalmid fishes to determine how these features, which are predicted to impact auditory capabilities, vary with respect to ecology and evolutionary history. We examined the two chambers of the swim bladder, the Weberian ossicles (tripus, intercalarium, and scaphium) and the otoliths of the sacculus (sagitta), lagena (asteriscus) and utriculus (lapillus). We used high resolution computed tomography (μCT) of museum specimens to measure the sizes of these structures among 20 species of serrasalmids from the three major clades of the family that were predicted to have potential functional consequences for the reception of sound. The acoustic environment of serrasalmid fishes from different habitats, such as slow-moving rivers and areas associated with rapids and waterfalls, may have influenced the evolution of the auditory system. Further, the evolution of hearing morphology in serrasalmids may be influenced by variation in environmental sounds that may act as feeding cues for species with different feeding ecologies. Thus, we tested for differences among ecological groups in the sizes of otoliths, swim bladder chambers, and Weberian ossicles relative to body length and used phylogenetic generalized least squares models to account for and measure the strength of phylogenetic signal in these relationships.

2 | MATERIALS AND METHODS

2.1 | Phylogeny

Our study includes 42% of the identified species in the most complete phylogenetic study (Thompson et al., 2014). These data represent 12 of the 16 genera in the family (Nelson et al., 2016). This sample captures much of the available deeper phylogenetic variation from the Thompson et al. (2014) phylogenetic hypothesis, for which the three major clades were hypothesized to have radiated between about 68–54 mya. Taxa from the Thompson et al. (2014) phylogeny that are not represented in our data set represent relatively recent radiations from taxa included in our study (mean branch lengths of nonsampled species to species in our study were 13 mya, 95% ≤ 29 mya). All taxa in our dataset are not present in a single published phylogeny. We constructed a phylogenetic hypothesis (Figure 1) based on the time calibrated molecular phylogeny (mt control region +10 nuclear genes) of Thompson et al. (2014), the mt control region and 12S and 16S rRNA phylogenies of Ortí, et al. (2008), and the mt cytochrome c oxidase subunit I gene phylogeny of *Tometes* spp. from Andrade et al. (2017). We constructed a matrix with rate parsimony (mrp; Bininda-Emonds, 2014; Sanderson, Purvis, & Henze, 1998) composite tree from three phylogeny nexus files that were constructed based on these published phylogenies. Nexus files were pruned to include only taxa that were present in our current study. Two species in our dataset, *Metynnis lippincottianus* and *Serrasalmus elongatus*, were not present in any of the available phylogenies. Based on taxonomy, we placed *M. lippincottianus* sister to its only congener in our dataset, *M. hypsauchen*, and likewise placed *S. elongatus* sister to *S. rhombeus* in a nexus file made based on the Thompson et al. (2014) phylogeny. We assembled a second nexus file based largely on data from the Ortí, et al. (2008). This study used a variable mtDNA marker that provided strong resolution of shallow nodes, but greater uncertainty for deep nodes (Ortí et al., 2008). Thus, we retained the topology of the “pacu,” “*Myleus*-pacu”, and “piranha” clades sensu Ortí et al. (2008) and Thompson et al. (2014). We used the pacu clade topology that was obtained by Ortí et al. (2008, Figure 2) from Bayesian inference of 12S and 16S mtDNA sequences and 58 indel characters, and which had the strongest bootstrap support for these taxa in that study. We used the *Myleus*-pacu clade topology obtained by Ortí et al. (2008, Figure 3) from Bayesian inference of control region mt DNA sequences (1,180 bp) and 119 indel characters, and which provided the strongest bootstrap support and provided the most resolution for these taxa in their study. We used the piranha clade topology obtained from Bayesian inference of the control region (1,130 bp) and 86 indel characters in Ortí et al. (2008, Figure 4). The third nexus file was constructed from the maximum likelihood tree obtained from two mitochondrial genes (COI and control region) in Andrade et al. (2017).

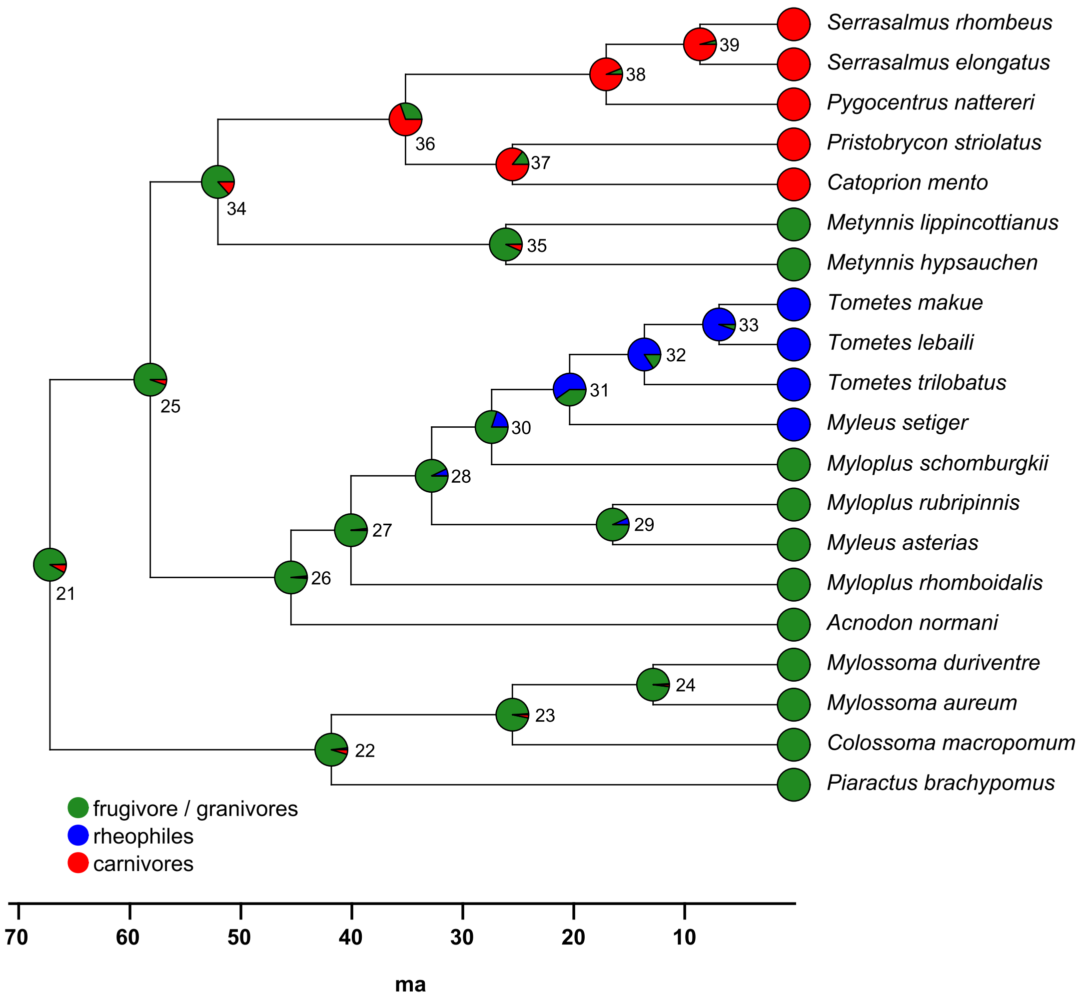

FIGURE 1 Phylogenetic hypothesis of serrasalmid fishes from our study. Composite tree based on Ortí et al. (2008), Thompson et al. (2014), and Andrade, Machado, Jégu, Farias, and Giarrizzo (2017) (see materials and methods for complete details). Branch length data are in millions of years (ma). Ecological groups are indicated by colors. Pie graphs at nodes depict ecological group ancestral state reconstruction (Bayesian MCML probabilities; Felsenstein, 2012; Revell, 2014, 2017). Probability values are given for each numbered node in Supporting Information Table S2

The phylogeny nexus files were transformed into a "multiPhylo" object using the "c.phylo" function in the "APE" library (Paradis et al., 2015) in R (R Core Team, 2014) and a mrp supertree was constructed from these phylogenies using the "mrp.supertree" function in the "phytools" R library (Revell, 2017) using the method "pratchet" and 10,000 iterations. Branch length estimates were only available for taxa and nodes present in the Thompson et al. (2014) phylogeny. In our phylogenetic hypothesis, we retained branch length estimates from Thompson et al. (2014) and estimated branch lengths of the other clades by evenly dividing the evolutionary time of the most recent calibrated node among the remaining branch steps evenly (Aquino & Colli, 2017). Branch length values were added to the nexus file in MESQUITE (Maddison & Maddison, 2017).

2.2 | Specimens, µCT-scans, and 3D-reconstructions

Specimens from the study were obtained from the Ichthyology Collection at the Muséum National d'Histoire Naturelle, Paris, France (MNHN; Supporting Information Table S1). Sample sizes for each species in this study ranged from one to four individuals (median of three). Sex of the individual fish used in this study was not known and we did not investigate potential sexual dimorphism in this study. We used µCT-scanning to produce 3D surfaces of the neurocranium, vertebrae 1–4, the neural complex, the Weberian ossicles, the otoliths, and the swim bladder (Figure 2). Specimens were scanned at the at the AST-RX technical platform at the MNHN with a µCT-scanner (v|tome|x 240 L, GE Sensing & Inspection Technologies phoenix|x-ray) with voltages between 85 and 130 V (mean 102) and current between 230 and 360 µA (mean 309). Scanning resulted in 1905 to 2023 slices per specimen (mean 2000). Two specimens of similar size were scanned at a time with the aim of producing similar resolution scans for the structures of interest. This resulted in isotropic voxel sizes that ranged from 31.1 to 100.0 µm (mean 53.6) and in terms of voxels/body length (standard length) ranged from 1,550 to 3,239 (mean 2,510). Segmentation and surface generation were performed using AVIZO 7.0 (FEI™, Mérignac, France). Semiautomatic thresholding was used to identify bones and otoliths. Individual bones were separated manually using a drawing tablet and the swim bladder tunic was segmented by hand. Surface generation of bones and otoliths was performed using the "constrained smoothing" setting. Additional smoothing was performed on the anterior and posterior swim bladder chambers to reduce noise from manual segmentation: unconstrained smoothing was performed and then a "smoothed surface" was

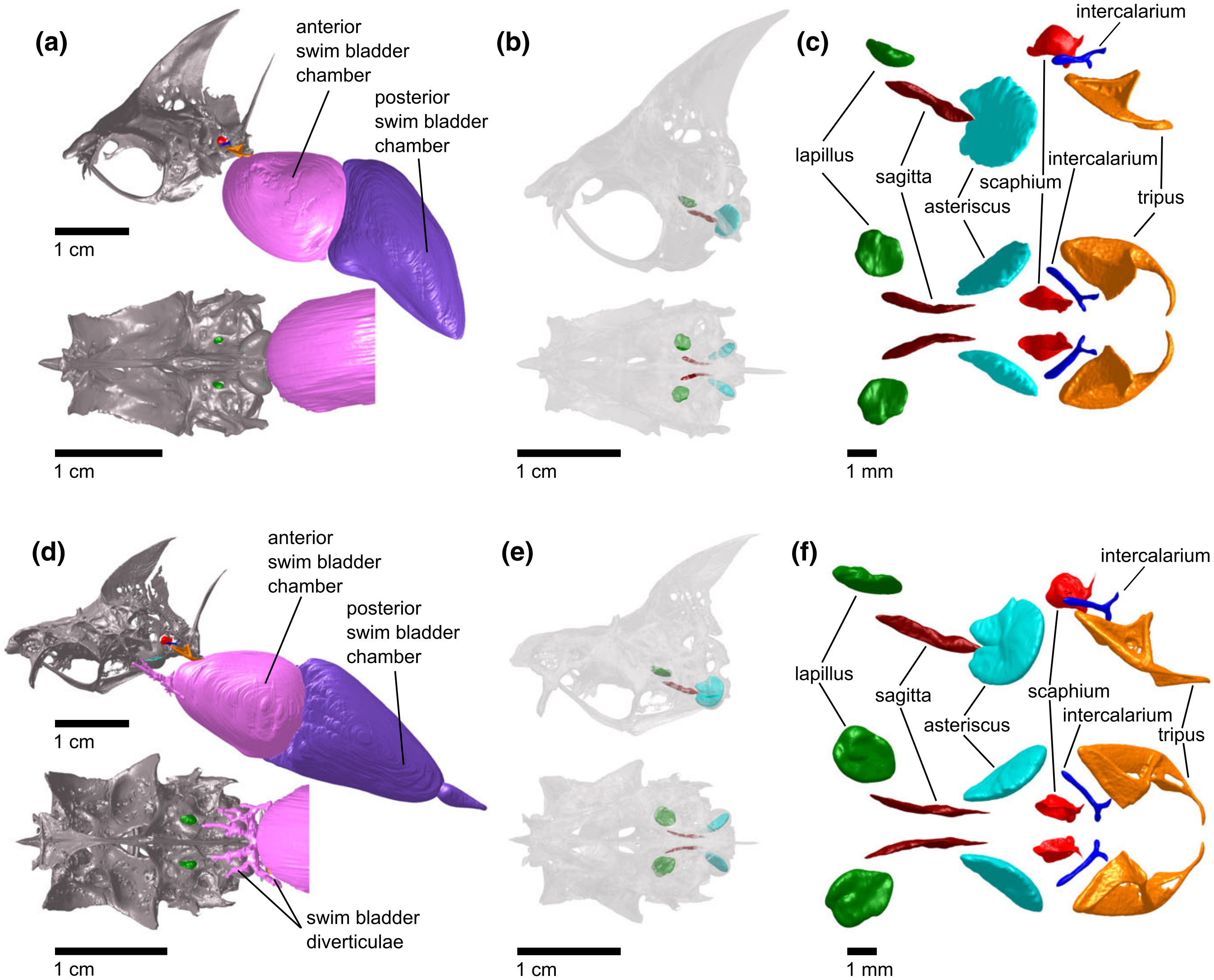

FIGURE 2 Surface reconstructions of the neurocranium, vertebrae 1–4, neural complex, Weberian ossicles, and swim bladder of (a, b, c) *Catoprion mento* (MNHN IC.1998–0312, 109 mm SL) and (d, e, f) *Mylossoma aureum* (MNHN IC.1998–0181, 108 mm SL). (a, d) left lateral view (top) of the neurocranium, vertebrae 1–4, Weberian ossicles, and swim bladder chambers and ventral view (bottom) of the neurocranium and anterior swim bladder. (b, e) left lateral (top) and ventral view (bottom) of a transparent rendering of the neurocranium showing the position of the otoliths (color) in the neurocranium. (c, f) left lateral (top) and dorsal views (bottom) of the Weberian ossicles (scaphium, intercalarium, tripus) and otoliths (utricular otolith = lapillus, saccular otolith = sagitta, lagenar otolith = lapillus). Note the lapillus is visible through the prootic foramen in the ventral view of the neurocranium (a, d). note the presence of anterior diverticulae, which project anteriorly (ventral to the basioccipital and prootic of the neurocranium) and posteriorly (lateral to the anterior end of the swim bladder). These diverticulae are present in both species of *Mylossoma* examined. SL = standard length

produced with 20 iterations and lambda of 0.6. Surfaces were exported as (.PLY) format files. Surface files were cleaned to fill-in holes, remove creased edges, and spikes with Geomagic Studio software (Geomagic Studio; Raindrop Geomagic, Research Triangle Park, NC). This post-processing removed these rare artifacts and visual inspection of the surface files indicated that structures were of comparable detail among individual specimens. Size measurements of the bilateral structures in this study (otoliths and Weberian ossicles) were made from the specimen's left side. The lagenar otoliths of several fish specimens were cracked. In one of these cases, the specimen was not used and in two other cases only the left otolith was cracked and the right otolith was analyzed instead (Supporting Information Table S1). Data from the anterior swim bladder chamber of one specimen were not included because of deflation and damage and data from the posterior swim bladder chamber of four individuals were not included because of deflation or damage (2 instances) or because CT-scan images did not extend far enough caudally (2 instances; Supporting Information Table S1).

2.3 | Ecological groups

Fish species were categorized into three broad ecological groups for analysis based on published accounts of feeding habits and habitat associations (Supporting Information Table S1). These ecological groups were (a) carnivores, species that mainly consume fish prey either whole or in pieces (muscle tissue, lepidophagy, fins); (b) frugivore/granivores, species that consume a substantial portion of fruits and seeds (more

FIGURE 3 Surface reconstructions of the otoliths and Weberian ossicles of a representative specimen from 20 serrasalmid species illustrate variation of the relative sizes of structures. For each species, a left lateral view of the left otoliths and ossicles is shown (top) and a dorsal view of both left and right otoliths and ossicles with anterior to the left (bottom) are shown. All structures are shown on the same scale relative to body length. Structure colors: Green = lapillus, brown = sagitta, light blue = asteriscus, red = scaphium, blue = intercalarium, yellow = tripus. For each specimen, the ecological group, major clade, genus/species, and length and MNHN catalog number are given (top to bottom). SL = standard length

than 25% frequency of occurrence in literature reports and in most cases >80%); and (c) rheophiles, species that are restricted in habitat to high-flow environments among rapids and waterfalls and that have a largely herbivorous diet composed of submerged plants of the family Podostomaceae.

Ancestral state reconstruction of discrete ecological groups (frugivore/granivores, rheophliles, and carnivores) was conducted with the "ancThresh" function of the R package phytools (Revell, 2014, 2017). This procedure utilizes Bayesian Markov Chain Monte Carlo (MCMC) analyses and a threshold model to determine thresholds associated

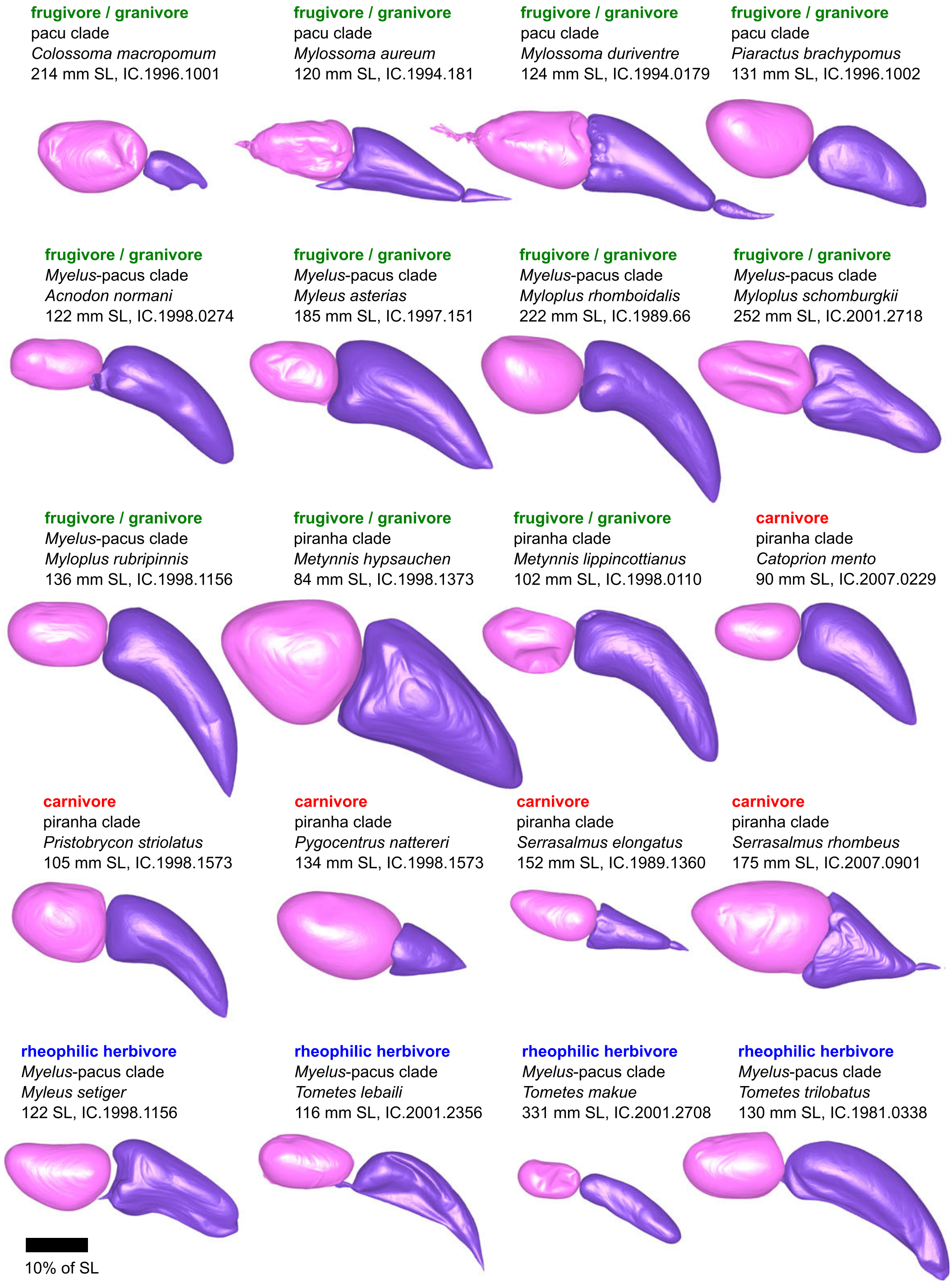

FIGURE 4 Surface reconstructions of the swim bladders of representative specimens from 20 serrasalmid species illustrate variation of the relative sizes of structures. The swim bladder is shown in left lateral view. The anterior swim bladder chamber is shown in pink and the posterior chamber is shown in purple. Swim bladders are shown on the same scale relative to body length. For each specimen, the ecological group, major clade, genus/species, and length and MNHN catalog number are given (top to bottom). SL = standard length

with the change of discrete characters between states (Felsenstein, 2012). The threshold model assumes that discrete states have a continuous underlying liability and when the liability exceeds a threshold, the character state of the discrete trait is observable (Felsenstein, 2012). For this procedure we ran 10^6 generations, with 200,000 burn-in generations, and a Brownian motion model was used.

2.4 | Size relationships with ecology

Fish specimens were measured for standard length (SL) by ruler to the nearest mm (Supporting Information Table S1). Volumes (all otoliths and Weberian ossicles) and surface areas (all otoliths) were calculated in Geomagic Studio. The linear distance between the anterior and posterior extremes of the sagitta was also measured in Geomagic Studio. Swim bladder chamber volumes and surface areas were estimated by measuring the size of the anterior swim bladder chamber and posterior swim bladder chamber surface files in Avizo. Size data were $\log_{10}$-transformed and species means were calculated for these size variables.

Size—ecology relationships were tested with phylogenetic generalized least squares (PGLS; Martins & Hansen, 1997; Symonds & Blomberg, 2014). We used this approach to test for the strength of the phylogenetic signal in the relationship between structure size and body size, and to simultaneously test for an effect of ecological group while accounting for phylogenetic signal. For these analyses, we tested the species mean of $\log_{10}$ size (volume, length, surface area, see above) of structures vs. the mean $\log_{10}$ SL and ecology was included as a factor in these models. We initially tested models that included interaction terms between body length (SL) and ecological group. When interaction terms were not significant ($p > .05$), we reported results from simplified PGLS models that did not include interactions. PGLS tests were conducted with the "caper" library (Orme et al., 2015) in R (R Core Team, 2014). In our PGLS models, δ (change in evolutionary rates) and κ (gradual vs. punctuated evolution) were fixed at 1 (assumption of Brownian motion for both). We used a maximum likelihood (ML) approach to determine the strength ($0 \leq \lambda \leq 1$) of the branch length phylogenetic signal λ: $\lambda = 0$, no phylogenetic signal and $\lambda = 1$. We used likelihood ratio tests to determine if significant differences existed between the ML estimate and null models of λ ($\lambda = 0$ and $\lambda = 1$; Freckelton, Harvey, & Pagel, 2002). In cases where the ML estimate of λ was not significantly different from $\lambda = 0$ or $\lambda = 1$, we also reported the PGLS models with these λ values. We considered the nonindependence of otoliths, Weberian ossicles, and swim bladder size related features using a sequential Bonferroni to adjust the type I error rate (Rice, 1989) to account for these 14 separate PGLS tests. For simplicity and clarity in text throughout, we refer to observed differences in sizes relative to body length (SL).

3 | RESULTS

Ancestral state reconstruction of ecological groups indicated that frugivory/granivory is likely an ancestral trait in extant serrasalmids and frugivore/granivores are present in all three clades (Figure 1). Rheophilic benthic herbivory and carnivory most likely evolved once, with rheophily present as a monophyletic subclade within the *Myleus*-pacu clade and carnivory as a derived trait in the piranha clade (Figure 1).

The body size range of fish in our study ranged from 63 to 252 mm standard length (Table 1). Otolith sizes among specimens were variable in terms of both absolute (Table 2) and relative size (Figure 3). Broad variation in absolute and relative size was also observed for the anterior and posterior chambers of the swim bladder (Table 3, Figure 4) and the Weberian ossicles (Table 4, Figure 3).

All structures examined were strongly correlated with body size (Tables 5–9, and Figures 5–7). Initial p-values from all PGLS F-tests were low (all $p < .001$) and the sequential Bonferroni retained $p \leq .05$ for hypothesis tests after accounting for multiple tests.

TABLE 1 Species and authority, abbreviations used, ecological group, number of specimens, and body size range of serrasalmids observed in this study

Species and authority	Abbreviation	Ecological group	N	SL (mm) $\bar{x}$	(range)
Acnodon normani Gosline	Ano	f	3	110	(104–122)
Catoprion mento (Cuvier)	Cme	c	3	91	(79–105)
Colossoma macropomum (Cuvier)	Cma	f	2	166	(118–214)
Metynnis hypsauchen (Müller & Troschel)	Mhy	f	3	90	(84–102)
Metynnis lippincottianus (cope)	Mli	f	4	105	(96–112)
Myleus asterias (Müller & Troschel)	Mas	f	2	192	(185–198)
Myleus setiger Müller & Troschel	Mse	r	3	105	(92–122)
Myloplus rhomboidalis (Cuvier)	Mrh	f	2	156	(89–222)
Myloplus rubripinnis (Müller & Troschel)	Mru	f	2	138	(136–140)
Myloplus schomburgkii (Jardine)	Msc	f	2	247	(242–252)
Mylossoma aureum (Spix & Agassiz)	Mau	f	2	114	(108–120)
Mylossoma duriventre (Cuvier)	Mdu	f	3	123	(118–127)
Piaractus brachypomus (Cuvier)	Pbr	f	3	146	(131–168)
Pristobrycon striolatus (Steindachner)	Pst	c	3	86	(63–104)
Pygocentrus nattereri Kner	Pna	c	3	136	(120–155)
Serrasalmus elongatus Kner	Sel	c	4	106	(63–152)
Serrasalmus rhombeus (Linnaeus)	Srh	c	4	177	(172–180)
Tometes lebaili Jégu, Keith & Belmont-Jégu	Tle	r	2	122	(116–128)
Tometes makue Jégu & dos Santos	Tma	r	3	206	(178–221)
Tometes trilobatus Valenciennes	Ttr	r	1	130	130

Abbreviations: c = carnivores; eco = ecological; f = frugivore/granivores; r = rheophilic herbivores; SL = standard length

TABLE 2 Mean and range of serrasalmid otolith sizes observed in this study

Species	Sagitta						Asteriscus				Lapillus			
	Length (mm) $\bar{x}$ (range)		Volume (mm^3) $\bar{x}$ (range)		Area (mm^2) $\bar{x}$ (range)		Volume mm^3 $\bar{x}$ (range)		Area (mm^2) $\bar{x}$ (range)		Volume mm^3 $\bar{x}$ (range)		Area (mm^2) $\bar{x}$ (range)	
Ano	3.4	(3.2–3.7)	0.3	(0.3–0.4)	7.9	(7.6–8.0)	7.6	(6.5–8.7)	27.3	(23.6–31.0)	1.4	(1.4–1.5)	7.9	(7.6–8.0)
Cme	3.1	(2.4–3.5)	0.1	(0.1–0.2)	3.8	(2.8–5.6)	2.0	(1.1–3.3)	12.1	(9.0–16.9)	0.5	(0.3–0.8)	3.8	(2.8–5.5)
Cma	7.6	(7.5–7.8)	0.9	(0.9–1.0)	19.6	(15.6–23.6)	13.4	(10.5–16.6)	52.9	(52.8–52.9)	4.5	(2.9–6.1)	19.6	(15.6–23.6)
Mhy	4.6	(3.8–5.4)	0.5	(0.3–0.6)	7.2	(5.3–9.1)	8.2	(5.0–12.2)	28.3	(21.7–37.2)	1.4	(0.8–1.9)	7.2	(5.3–9.1)
Mli	4.5	(3.9–5.2)	0.4	(0.3–0.5)	7.3	(6.2–8.5)	7.4	(4.8–10.8)	26.3	(20.7–34.0)	1.0	(1.0–1.0)	6.3	(6.2–6.3)
Mas	6.1	(5.7–6.6)	1.5	(1.4–1.6)	21.5	(20.9–22.0)	18.8	(16.5–21.0)	53.2	(49.7–56.7)	6.4	(6.1–6.7)	21.5	(20.9–22.0)
Mse	3.2	(2.9–3.5)	0.2	(0.1–0.2)	6.1	(5.4–7.2)	1.4	(1.2–1.7)	10.1	(9.0–11.8)	0.9	(0.8–1.2)	6.1	(5.4–7.2)
Mrh	4.4	(3.4–5.4)	0.4	(0.3–0.5)	13.6	(7.3–20.0)	6.6	(2.1–11.0)	24.1	(12.3–35.9)	3.7	(1.4–6.0)	13.6	(7.3–20.0)
Mru	4.8	(4.5–5.1)	0.6	(0.5–0.6)	14.8	(13.7–16.0)	8.3	(7.3–9.2)	30.6	(28.3–33.0)	3.6	(3.2–4.0)	14.8	(13.7–15.9)
Msc	6.6	(6.2–7.0)	1.5	(1.0–1.9)	27.5	(27.0–27.9)	25.3	(21.1–29.5)	75.9	(70.8–80.9)	8.1	(7.6–8.6)	27.4	(27.0–27.9)
Mau	4.6	(4.5–4.8)	0.5	(0.3–0.6)	10.0	(9.3–10.8)	4.7	(4.2–5.3)	21.1	(20.0–22.2)	1.9	(1.6–2.1)	10.0	(9.3–10.7)
Mdu	5.6	(5.5–5.9)	0.7	(0.7–0.7)	13.5	(13.2–13.9)	7.3	(6.5–7.7)	27.7	(25.3–29.2)	3.1	(2.9–3.3)	13.5	(13.2–13.9)
Pbr	6.0	(5.8–6.1)	0.5	(0.4–0.7)	9.7	(8.3–10.9)	5.3	(4.1–7.0)	26.9	(24.5–31.7)	1.8	(1.3–2.2)	9.7	(8.3–10.9)
Pst	3.1	(1.8–4.0)	0.2	(0.0–0.2)	4.1	(2.1–5.6)	2.2	(0.8–3.5)	12.2	(6.3–16.9)	0.6	(0.2–0.9)	4.1	(2.1–5.6)
Pna	4.2	(4.0–4.5)	0.3	(0.3–0.4)	19.0	(17.4–20.7)	7.9	(6.7–8.8)	31.9	(28.6–33.6)	5.3	(4.4–5.9)	19.0	(17.4–20.7)
Sel	4.0	(2.5–6.2)	0.3	(0.1–0.8)	5.8	(2.5–13.1)	4.2	(0.6–11.7)	17.2	(4.8–38.5)	1.1	(0.2–3.3)	5.8	(2.5–13.1)
Srh	5.9	(4.4–7.4)	0.5	(0.2–0.6)	15.9	(10.7–19.5)	13.8	(7.0–19.1)	45.3	31.2–55.7)	4.5	(2.4–5.9)	15.8	(10.7–19.5)
Tle	3.0	(2.9–3.1)	0.2	(0.2–0.2)	6.7	(6.5–6.9)	1.4	(1.3–1.5)	9.5	(9.4–9.6)	1.2	(1.1–1.2)	6.7	(6.5–6.9)
Tma	4.8	(4.0–5.4)	0.8	(0.5–1.0)	10.7	(9.3–12.0)	4.5	(3.2–5.6)	21.1	(16.4–24.0)	2.4	(1.9–2.8)	10.7	(9.3–12.0)
Ttr	4.0		0.4		9.2		2.5		14.2	(14.2–14.2)	1.7		9.2	

Abbreviation: SL = standard length. Species abbreviations follow Table 1.

TABLE 3 Mean and range of serrasalmid swim bladder chamber sizes observed in this study

Species	Anterior sb chamber volume cm^3 $\bar{x}$ (range)		Anterior sb chamber Area (mm^2) $\bar{x}$ (range)		Posterior sb chamber volume mm^3 $\bar{x}$ (range)		Posterior sb chamber Area (mm^2) $\bar{x}$ (range)	
Ano	0.9	(0.6–1.2)	5.3	(4.4–6.6)	1.2	(0.3–2.2)	7.5	(4.2–11.2)
Cme	0.8	(0.4–1.5)	4.7	(3.3–7.4)	1.0	(0.5–1.5)	6.1	(4.3–8.4)
Cma	11.2	(11.0–11.5)	28.0	(26.6–29.4)	1.1	(1.1–1.1)	8.4	(8.4–8.4)
Mhy	1.2	(0.6–2.1)	6.0	(3.7–9.1)	1.4	(0.9–2.3)	7.9	(5.5–11.9)
Mli	1.1	(0.6–1.6)	6.2	(4.3–7.7)	1.7	(1.4–2.0)	9.0	(7.6–10.4)
Mas	5.0	(4.6–5.4)	16.4	(14.6–18.2)	13.0	(12.7–13.4)	33.1	(32.6–33.7)
Mse	1.2	(0.8–1.7)	5.9	(4.8–7.6)	0.8	(0.5–1.4)	6.5	(4.8–9.1)
Mrh	8.1	(1.2–15.1)	18.7	(6.1–31.3)	10.3	(1.0–19.5)	26.7	(7.8–45.6)
Mru	3.2	(2.8–3.6)	11.2	(10.4–12.1)	4.4	(4.2–4.7)	17.2	(16.7–17.6)
Msc	19.8	(18.5–21.2)	53.7	(40.1–67.2)	18.0	(14.6–21.3)	58.3	(51.9–64.7)
Mau	1.4	(1.2–1.5)	7.6	(7.2–7.9)	1.3	(1.1–1.5)	8.9	(8.1–9.7)
Mdu	1.9	(0.9–2.8)	10.1	(8.9–11.8)	2.3	(2.2–2.4)	11.9	(11.4–12.5)
Pbr	3.2	(2.8–4.1)	12.0	(11.1–13.8)	1.6	(1.6–1.6)	9.7	(9.7–9.8)
Pst	0.9	(0.4–1.4)	5.0	(3.0–6.4)	0.6	(0.2–1.2)	4.3	(2.2–7.1)
Pna	4.4	(3.7–4.9)	14.4	(12.9–15.2)	0.6	(0.4–0.8)	5.2	(3.4–6.2)
Sel	1.6	(0.2–4.4)	6.9	(1.9–14.8)	0.9	(0.1–1.9)	6.2	(1.2–10.5)
Srh	9.3	(5.4–12.9)	24.1	(17.0–29.3)	2.8	(1.7–4.4)	14.0	(9.6–19.5)
Tle	1.0	(0.9–1.0)	5.5	(5.4–5.5)	0.8	(0.8–0.8)	6.5	(6.5–6.5)
Tma	5.9	(3.3–7.3)	18.6	(12.6–22.2)	6.9	(3.3–10.7)	27.1	(17.7–32.0)
Ttr	2.0		8.8		2.6		16.6	

Abbreviation: SL = standard length. Species abbreviations follow Table 1.

3.1 | Sagitta (saccular otolith) size

The relationship between sagitta length and body size among species was influenced by phylogeny and did not differ among ecological groups. ML PGLS analysis of sagitta length indicated significant phylogenetic signal ($\lambda > 0$) and the ML model ($\lambda = 0.89$) was intermediate between $\lambda = 0$ and Brownian motion (Table 5, Figure 5a). Sagitta

TABLE 4 Mean and range of serrasalmid Weberian ossicle sizes observed in this study

Species	Tripus volume (mm^3) $\bar{x}$ (range)		Intercalarium volume (mm^3) $\bar{x}$ (range)		Scaphium volume (mm^3) $\bar{x}$ (range)	
Ano	1.42	(1.04–2.10)	0.26	(0.16–0.45)	0.37	(0.29–0.42)
Cme	0.79	(0.39–1.47)	0.06	(0.04–0.09)	0.19	(0.09–0.36)
Cma	9.26	(8.77–9.74)	0.93	(0.92–0.93)	1.68	(1.60–1.75)
Mhy	1.27	(0.54–2.19)	0.11	(0.05–0.17)	0.30	(0.16–0.52)
Mli	1.26	(0.98–1.53)	0.08	(0.07–0.10)	0.37	(0.24–0.48)
Mas	8.12	(6.34–9.91)	0.76	(0.67–0.84)	2.06	(1.68–2.44)
Mse	1.16	(0.61–1.66)	0.12	(0.06–0.18)	0.24	(0.14–0.34)
Mrh	8.17	(0.91–15.43)	0.69	(0.07–1.32)	1.60	(0.17–3.03)
Mru	3.09	(2.86–3.31)	0.26	(0.24–0.27)	0.66	(0.53–0.79)
Msc	29.40	(27.04–31.77)	1.80	(1.59–2.02)	5.02	(4.73–5.32)
Mau	1.72	(1.57–1.87)	0.12	(0.10–0.15)	0.41	(0.32–0.51)
Mdu	3.58	(2.36–4.32)	0.22	(0.17–0.24)	0.67	(0.44–0.78)
Pbr	4.12	(2.74–6.60)	0.50	(0.22–0.84)	0.88	(0.51–1.31)
Pst	1.10	(0.39–1.71)	0.06	(0.02–0.08)	0.19	(0.06–0.37)
Pna	6.82	(3.93–9.40)	0.47	(0.36–0.59)	0.99	(0.62–1.31)
Sel	2.09	(0.27–4.94)	0.13	(0.02–0.28)	0.35	(0.08–0.77)
Srh	12.20	(11.38–13.15)	0.94	(0.79–1.17)	2.37	(2.04–2.67)
Tle	1.97	(1.74–2.20)	0.15	(0.11–0.18)	0.30	(0.26–0.34)
Tma	7.88	(3.95–11.10)	0.69	(0.46–0.83)	2.09	(1.22–2.60)
Ttr	1.84		0.12		0.32	

Abbreviation: SL = standard length. Species abbreviations follow Table 1.

TABLE 5 Results from PGLS and maximum likelihood (ML) tests of λ for models that investigated covariation between sagitta size (length, volume, and surface area) and body length for 20 serrasalmid species

Model, ML test Model terms	d.f.	F-stat	Est.	SE	*t*-val.	*p*	r^2 adj	Log likelihood	AICc
Sagitta length									
λ 95% CI: 0.12–1.00									
λ = 0? *p* = **.025**									
λ = 1? *p* = .630									
ML λ = .89	3,16	26.89				**<.001**	0.804	31.20	−54.39
Intercept			−0.93	0.201	−4.63	**<.001**			
SL			0.74	0.094	7.82	**<.001**			
f vs. c			0.07	0.055	1.33	.200			
r vs. c			−0.01	0.068	−0.20	.847			
r vs. f			−0.09	0.045	−1.94	.071			
Sagitta volume									
λ 95% CI: 0.00–1.00									
λ = 0? *p* = 1.00									
λ = 1? *p* = **.006**									
ML λ = 0.00	3,16	39.89				**<.001**	0.860	16.82	
Intercept			−4.11	0.459	−9.24	**<.001**			
SL			1.73	0.222	7.78	**<.001**			
f vs. c			0.32	0.066	4.94	**<.001**			
r vs. c			0.05	0.080	0.62	.541			
r vs. f			−0.27	0.068	−4.02	**<.001**			
Sagitta area									
λ 95% CI: 0.00–0.78									
λ = 0? *p* = .508									
λ = 1? *p* = .150									
ML λ = 0.59	3,16	33.21				**<.001**	0.862	22.77	−37.54
Intercept			−1.97	0.329	−6.00	**<.001**			
SL			1.23	0.157	7.85	**<.001**			
f vs. c			0.21	0.069	3.09	**.007**			
r vs. c			0.02	0.085	0.20	.843			
r vs. f			−0.20	0.061	−3.20	**.006**			

Abbreviations: c = carnivores, Est. = estimate, f = frugivore / granivores, r = rheophilic, SL = standard length. For comparisons among ecological groups, coefficient estimates indicate the magnitude and relative relationship of the groups (e.g., f vs. c, when positive indicates frugivores are relatively higher for that size trait than carnivores).

length was not associated with ecological group (Table 4, Supporting Information Table S2).

The relationship between sagitta volume and body size among species did not appear to be influenced by phylogeny and differences in sagitta volume were observed among ecological groups. ML PGLS analysis of sagitta volume indicated significant deviation from $\lambda = 1$ (Brownian motion) and indicated no phylogenetic signal (ML $\lambda = 0$; Table 5). Sagitta volume in frugivore/granivores was larger compared to carnivores and rheophiles in the $\lambda = 0$ model (Figure 5b, Table 5).

The relationship between sagitta surface area and body size among species may have been influenced by phylogeny and differences in sagitta surface area were observed among ecological groups. ML PGLS analysis of sagitta surface area indicated that λ did not differ significantly from 0 or 1, however, ML favored $\lambda = 0.59$ (Table 5). Sagitta area in frugivore/granivores was greater compared to carnivores (Figure 5c, Table 5, Supporting Information Table S2). In addition, sagitta area in frugivore/granivores was greater compared to both carnivores and rheophiles in ML and $\lambda = 0$ models (Figure 5c, Table 5, Supporting Information Table S2).

3.2 | Asteriscus (lagenar otolith) size

The relationship between asteriscus volume and body size among species did not appear to be influenced by phylogeny and asteriscus volume of frugivore/granivore species and carnivores varied at different rates relative to body size. ML PGLS analysis of asteriscus volume did not indicate significant phylogenetic signal and ML favored $\lambda = 0$ (Table 6). A significant interaction term indicated a difference between the asteriscus volume-body length scaling relationship for the frugivore/granivore and carnivore ecological groups in the ML $\lambda = 0$ model. Asteriscus volume scaled at a higher rate to body size in carnivores (Figure 5d, Table 6). The frugivore/granivore *y*-intercept was greater than carnivores, indicating larger asteriscus size in frugivore/

TABLE 6 Results from PGLS and maximum likelihood (ML) tests of λ for models that investigated co-variation between asteriscus size (volume and surface area) and body length for 20 serrasalmid species

Model, ML test Model terms	d.f.	*F*-stat	Est.	SE	*t*-val.	*p*	r^2 adj	Log likelihood	AICc
Asteriscus volume									
λ 95% CI: 0.00–1.00									
λ = 0? *p* = 1.000									
λ = 1? *p* = .289									
ML λ = 0.00	5,14	22.01				**<.001**	0.847	14.09	−16.19
Intercept			−5.33	1.094	−4.88	**<.001**			
SL			2.88	0.532	5.42	**<.001**			
f vs. c			3.23	1.342	2.41	**.003**			
r vs. c			1.86	1.778	1.04	.314			
r vs. f			−1.38	1.604	−0.86	.405			
SL*f vs. c			−1.46	0.644	−2.27	**.040**			
SL*r vs. c			−1.09	0.845	−1.30	.216			
SL*r vs. f			0.37	0.751	0.49	.632			
Asteriscus area									
λ 95% CI: 0.00–1.00									
λ = 0? *p* = 1.00									
λ = 1? *p* = .052									
ML λ = 0.00	3,16	33.46				**<.001**	0.837	19.25	−30.51
Intercept			−1.62	0.408	−3.98	**.001**			
SL			1.41	0.197	7.15	**<.001**			
f vs. c			0.12	0.058	2.03	.060			
r vs. c			−0.27	0.071	−3.87	**.001**			
r vs. f			−0.39	0.060	−6.51	**<.001**			

Abbreviations: c = carnivores; Est. = estimate; f = frugivore/granivores; r = rheophilic; SL = standard length. For comparisons among ecological groups, coefficient estimates indicate the magnitude and relative relationship of the groups (e.g., f vs. c, when positive indicates frugivores are relatively higher for that size trait than carnivores).

granivores, at least in small bodied fish (Figure 5d, Table 6, Supporting Information Table S3).

The relationship between asteriscus surface area and body size among species did not appear to be influenced by phylogeny and differences in asteriscus surface area were observed among ecological groups. The ML PGLS model of asteriscus surface area did not differ significantly from $\lambda = 0$ or $\lambda = 1$, and ML favored $\lambda = 0$ (Table 6). Asteriscus surface area was significantly larger in frugivore/granivores (Figure 5e, Table 6, Supporting Information Table S3). In addition, asteriscus area was larger in carnivores compared to rheophiles in the ML $\lambda = 0$ model (Figure 5e, Table 6).

3.3 | Lapillus (utricular otolith) size

The relationship between lapillus volume and body size among species did not appear to be influenced by phylogeny and lapillus volume of frugivore/granivore species and carnivores varied at different rates relative to body size. ML PGLS analysis of lapillus volume diverged significantly from Brownian motion ($\lambda = 1$) and indicated no phylogenetic signal (ML $\lambda = 0$; Table 7). Interaction terms indicated significant differences in the scaling relationships between lapillus volume and body length between the frugivore/granivore and carnivore groups and between the rheophiles and carnivores (Table 7). Lapillus volume increases most rapidly with increasing body size in carnivores and most slowly with increasing body size in rheophiles (Figure 5f). Significant main effects indicated a lower *y*-intercept for carnivores, with smaller bodied carnivores having a smaller lapillus volume than frugivore/granivores and rheophiles (Table 7, Figure 5f).

The relationship between lapillus area and body size among species did not appear to be influenced by phylogeny and lapillus area of frugivore/granivore species and carnivores varied at different rates relative to body size. Relationships among ecological groups for lapillus area followed a similar trend to lapillus volume. ML PGLS analysis of lapillus surface area also diverged significantly from Brownian motion ($\lambda = 1$) and indicated no phylogenetic signal (ML $\lambda = 0$; Table 7). An interaction term indicated a significant difference between the scaling relationship of lapillus surface area and body size between rheophiles and carnivores (Table 7). Surface area increased at a greater rate relative to increasing body size in carnivores compared to rheophiles (Figure 5g). A significant main effect indicated a lower *y*-intercept for carnivores compared to rheophiles, with smaller bodied carnivores having a smaller lapillus size than rheophiles of comparable body size (Figure 5g).

3.4 | Swim bladder chamber size

The relationship between anterior chamber volume and body size among species did not appear to be influenced by phylogeny and

TABLE 7 Results from PGLS and maximum likelihood (ML) tests of λ for models that investigated covariation between lapillus size (volume and surface area) and body length for 20 serrasalmid species

Model, ML test Model terms	d.f.	*F*-stat	Est.	SE	*t*-val.	*p*	r^2 adj	Log likelihood	AICc
Lapillus volume									
λ 95% CI: 0.00–0.47									
λ = 0? *p* = 1.000									
λ = 1? *p* = **.003**									
ML λ = 0.00	5,14	19.38				**<.001**	0.829	12.72	−13.44
Intercept			−7.56	1.171	−6.45	**<.001**			
SL			3.72	0.570	6.53	**<.001**			
f vs. c			3.49	1.424	2.45	**.028**			
r vs. c			4.73	1.980	2.39	**.032**			
r vs. f			1.24	1.791	0.69	.501			
SL*f vs. c			−1.62	0.685	−2.37	**.033**			
SL*r vs. c			−2.32	0.941	−2.46	**.027**			
SL*r vs. f			−0.70	0.840	−0.83	.419			
Lapillus area									
λ 95% CI: 0.00–0.49									
λ = 0? *p* = 1.00									
λ = 1? *p* = **.004**									
ML λ = 0.00	5,14	19.35				**<.001**	0.828	20.47	−28.95
Intercept			−4.07	0.795	−5.13	**<.001**			
SL			2.40	0.387	6.21	**<.001**			
f vs. c			1.96	0.967	2.03	.062			
r vs. c			3.15	1.344	2.35	**.034**			
r vs. f			1.19	1.215	0.98	.343			
SL*f vs. c			−0.90	0.464	−1.94	.073			
SL*r vs. c			−1.54	0.639	−2.42	**.030**			
SL*r vs. f			0.65	0.570	−1.13	.277			

Abbreviations: c = carnivores, Est. = estimate, f = frugivore / granivores, r = rheophilic, SL = standard length. For comparisons among ecological groups, coefficient estimates indicate the magnitude and relative relationship of the groups (e.g., f vs. c, when positive indicates frugivores are relatively higher for that size trait than carnivores).

differences in anterior chamber volume were observed among ecological groups. ML PGLS analysis of anterior chamber volume indicated significant deviation from Brownian motion (λ = 1) models and indicated no phylogenetic signal (ML λ = 0; Table 8). Anterior chamber volume in carnivores was larger compared to rheophiles (Figure 6a, Table 8).

The relationship between anterior chamber surface area and body size among species did not appear to be influenced by phylogeny and differences in anterior chamber surface area were observed among ecological groups. ML PGLS analysis of anterior swim bladder chamber surface area indicated significant deviation from Brownian motion (λ = 1) model and indicated no phylogenetic signal (ML λ = 0; Table 8). Anterior chamber surface area was significantly smaller in rheophiles compared to both than carnivores and frugivore/granivores (Figure 6 (b), Table 8).

The relationship between posterior chamber volume and body size among species may have been influenced by phylogeny and differences were observed among ecological groups. In contrast to results from the anterior chamber, the largest variation among ecological groups for the posterior chamber was observed between frugivore/granivores and carnivores. ML PGLS analysis of posterior chamber volume indicated no significant deviation λ = 0 or λ = 1 and the ML model favored a value intermediate between no-phylogenetic signal and Brownian motion, λ = 0.28 (Table 9). Posterior chamber volume in frugivore/granivores was significantly larger compared to carnivores in the ML λ = 0.28 and λ = 0 models, but not the λ = 1 model (Figure 6c, Table 9, Supporting Information Table S4).

The relationship between posterior chamber surface area and body size among species was influenced by phylogeny and differences were observed among ecological groups. ML PGLS analysis of posterior chamber area indicated significant deviation from the Brownian motion (λ = 1) model and indicated no phylogenetic signal (ML λ = 0; Table 5). As was observed for posterior chamber volume, posterior chamber surface area was lowest for carnivores. Posterior chamber surface area in frugivore/granivores was significantly larger compared to carnivores (Figure 6d, Table 9).

3.5 | Weberian ossicle size

The relationship between tripus volume and body size among species did not appear to be influenced by phylogeny and differences in tripus volume were observed among ecological groups. ML PGLS analysis of

TABLE 8 Results from PGLS and maximum likelihood (ML) tests of λ for models that investigated co-variation between anterior swim bladder chamber size (volume and surface area) and body length for 20 serrasalmid species

Model, ML test Model terms	d.f.	*F*-stat	Est.	SE	*t*-val.	*p*	r^2 adj	Log likelihood	AICc
Anterior swim bladder volume									
λ 95% CI: 0.00–0.53									
λ = 0? *p* = 1.000									
λ = 1? *p* = **.006**									
ML *λ* = 0.00	3,16	40.12				**<.001**	0.861	10.43	−12.85
Intercept			−3.43	0.632	−5.42	**<.001**			
SL			3.26	0.306	10.64	**<.001**			
f vs. c			−0.07	0.090	−0.78	.448			
r vs. c			−0.25	0.111	−2.26	**.038**			
r vs. f			−0.18	0.094	−1.91	.074			
Anterior swim bladder area									
λ 95% CI: 0.00–0.81									
λ = 0? *p* = 1.00									
λ = 1? *p* = **.025**									
ML *λ* = 0.00	3,16	48.41				**<.001**	0.882	19.64	−31.27
Intercept			−1.68	0.399	−4.21	**<.001**			
SL			2.24	0.193	11.58	**<.001**			
f vs. c			−0.03	0.057	−0.51	.619			
r vs. c			−0.17	0.070	−2.41	**.028**			
r vs. f			−0.14	0.059	−2.36	**.031**			

Abbreviations: c = carnivores, Est. = estimate, f = frugivore / granivores, r = rheophilic, SL = standard length. For comparisons among ecological groups, coefficient estimates indicate the magnitude and relative relationship of the groups (e.g., f vs. c, when positive indicates frugivores are relatively higher for that size trait than carnivores).

tripus volume indicated significant divergence from a Brownian motion (*λ* = 1) model and indicated no phylogenetic signal (ML *λ* = 0; Table 10). Tripus volume in rheophiles was significantly smaller compared to both carnivores and frugivore/granivores (Figure 7(a), Table 10).

The relationship between intercalarium volume and body size among species did not appear to be influenced by phylogeny and differences in intercalarium volume were observed among ecological groups. ML PGLS analysis of intercalarium volume indicated significant divergence from a Brownian motion (*λ* = 1) model and indicated no phylogenetic signal (ML *λ* = 0; Table 10). As was observed for tripus volume, rheophiles generally had the smallest intercalarium volume. The intercalarium of rheophiles was significantly smaller than frugivore/granivores (Figure 7b, Table 10).

The relationship between scaphium volume and body size among species did not appear to be influenced by phylogeny and scaphium volume differed from the other Weberian ossicles in that there was evidence of variation in the scaling relationship with body size among ecological groups. ML PGLS analysis of scaphium volume indicated significant divergence from a Brownian motion (*λ* = 1) model and indicated no phylogenetic signal (ML *λ* = 0; Table 10). An interaction term indicated a significant difference between the scaling relationship of scaphium volume and body size between frugivore/granivores and carnivores (Table 10). Scaphium volume increased at a greater rate as a function of body size in carnivores compared to frugivore/granivores (Figure 7c). Scaphium volume in small bodied carnivores, however, was smaller than scaphium volume in comparably-sized frugivore/granivores, which was indicated by a significantly different y-intercept (Table 10, Figure 7c).

3.6 | Summary of structure sizes

An examination of surface reconstructions of comparably sized specimens (Figure 8) from different ecological groups shown at the same scale illustrates the general size trends observed in the PGLS analyses (Table 11). Frugivore/granivores tend to have a greater sagitta volume and surface area. Asteriscus volume and surface area are lowest for rheophiles (Figure 8), although the observed differences in volume in the PGLS analysis were not statistically significant, perhaps because of the broad variation in size among all species and the divergent scaling relationships of carnivores and frugivore/granivores. The small asteriscus sizes of rheophiles were accompanied by reduced lagenar bullae of the otic neurocranium (Figure 8), while frugivore/granivores and carnivores have large lagenar bullae that accommodate their large asteriscae (Figures 2 and 8). The anterior swim bladder volume is largest in carnivores and smallest for rheophiles (Figure 8), while anterior chamber surface area is greatest for both frugivore/granivores and carnivores. Frugivore/granivores in the genus *Mylossoma* have anterior diverticulae (Figure 2d) that extend below the neurocranium and posteriorly, medial to the pectoral girdle. These diverticulae increase the surface area of this chamber. Relative to body length, the size (volume and surface area) of the posterior swim bladder chamber is smaller in most carnivores (Figures 2 and 8) and largest in frugivore/granivores. Tripus volume of rheophiles is smaller than carnivores and

TABLE 9 Results from PGLS and maximum likelihood (ML) tests of λ for models that investigated covariation between posterior swim bladder chamber size (volume and surface area) and body length for 20 serrasalmid species

Model, ML test Model terms	d.f.	*F*-stat	Est.	SE	*t*-val.	*p*	r^2 adj	Log likelihood	AICc
Posterior swim bladder volume									
λ 95% CI: 0.00–1.00									
λ = 0? *p* = .650									
λ = 1? *p* = .075									
ML λ = 0.28	3,16	21.64				**<.001**	0.765	4.92	−1.84
Intercept			−2.66	0.818	−3.25	**.005**			
SL			2.70	0.394	6.86	**<.001**			
f vs. c			0.35	0.139	2.48	**.025**			
r vs. c			0.09	0.173	0.56	.586			
r vs. f			−0.25	0.135	−1.84	.084			
Posterior swim bladder area									
λ 95% CI: 0.00–0.95									
λ = 0? *p* = 1.00									
λ = 1? *p* = **.030**									
ML λ = 0.00	3,16	30.73				**<.001**	0.824	15.05	−22.10
Intercept			−1.21	0.497	−2.44	**.027**			
SL			1.94	0.240	8.08	**<.001**			
f vs. c			0.22	0.071	3.11	**.007**			
r vs. c			0.14	0.087	1.65	.119			
r vs. f			−0.08	0.074	−1.02	.324			

Abbreviations: c = carnivores, Est. = estimate, f = frugivore / granivores, r = rheophilic, SL = standard length. For comparisons among ecological groups, coefficient estimates indicate the magnitude and relative relationship of the groups (e.g., f vs. c, when positive indicates frugivores are relatively higher for that size trait than carnivores).

frugivore/granivores (Figure 8). Intercalarium volume was larger for frugivore/granivores than rheophiles (Figure 8).

4 | DISCUSSION

This study revealed relative size differences among the otoliths, the swim bladder, and the Weberian apparatus among serrasalmids that were related to the fishes' feeding ecologies. Phylogenetic signal in the relationship between structure size and body size was most evident for sagitta length, followed by sagitta area, posterior swim bladder surface area, and posterior swim bladder volume. Phylogenetic signal was not pronounced among the other otoliths and accessory auditory structures.

4.1 | Rheophiles differ from other serrasalmids

Differences among ecological groups were most pronounced for rheophiles associated with high energy environments that are likely to have increased background noise. These differences in size relative to body length included smaller otoliths: asteriscus surface area of rheophiles was smaller compared to of frugivore/granivores and carnivores; sagittae of rheophiles were generally smaller than sagittae of frugivore/granivores, and larger carnivores had larger relative lapilli sizes than rheophiles of comparable body size. Rheophiles had smaller anterior swim bladder chambers than frugivore/granivores and all three Weberian ossicles of rheophiles were smaller than the ossicles of the other ecological groups.

Environments with rapids are characterized by higher sound pressure levels across most frequencies, especially above 300 Hz (Lugli & Fine, 2003; Tonolla, Lorang, Heutschi, Gotschalk, & Tockner, 2011). The smaller Weberian ossicle size observed among rheophiles in this study is suggested to result in the reduction of the inertia of the ossicles (Chardon & Vandewalle, 1997). Cypriniform otophysan species living in rapids are characterized by a pronounced reduction in swim bladder size and a bony encapsulation that surrounds the swim bladder (Bird & Hernandez, 2007). A similar condition has been reported for some species of catfishes (Siluriformes; Lechner & Ladich, 2008). Reduced swim bladders and Weberian ossicles can be associated with a benthic mode-of-life (Chardon & Vandewalle, 1997), which appears to be a strategy for some of these other rheophilic otophysans. Rheophilic serrasalmids, by contrast, swim within the water column and thus require buoyancy from the swim bladder and lack the extremely reduced swim bladders of benthic rheophilic otophysans.

A rheophilic mode of life likely evolved once among serrasalmids (Figure 1). Rheophiles in this study were generally characterized by otoliths that were either relatively smaller or reduced in surface area. In otophysans, sagittae of the saccules are predicted to respond mainly to endolymphatic flow produced by indirect stimulation of the ears from Weberian ossicle movements (Chardon et al., 2003; Popper et al., 2003). Smaller sagittae may be able to respond to lower amplitude movements that result from the smaller sized Weberian ossicles. Perhaps there are size constraints of the set of these structures

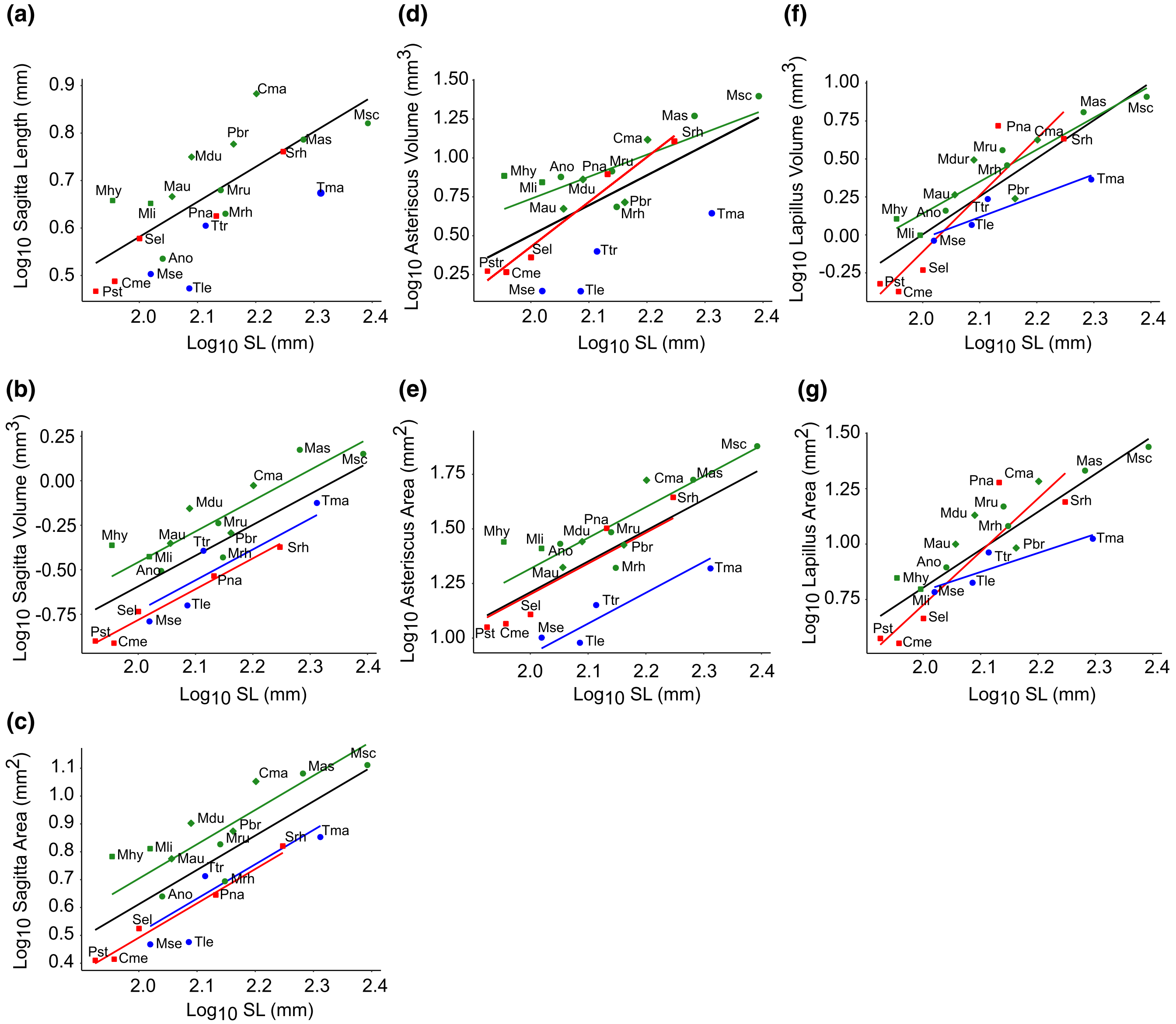

FIGURE 5 Relative otolith size relationships among serrasalmid species. (a) Sagitta (saccular otolith) length vs. standard length (SL), (b) sagitta volume vs. SL, (c) sagitta surface area vs. SL, (d) asteriscus (lagenar otolith) volume vs. SL, (e) asteriscus surface area vs. SL, (f) lapillus (utricular otolith) volume vs. SL, and (g) lapillus surface area vs. SL. Fitted lines determined from maximum likelihood λ PGLS models (Tables 4 and 6). Black lines are fits among all species. Colored lines present when significant differences (Tables 5 and 7) were observed among some or all ecological groups: Green = frugivore/carnivores, red = carnivores, blue = rheophiles. See Table 1 for species abbreviations

(ossicles + sagittae) in which movements can occur in these noisy environments with sufficient displacement to stimulate saccular hair cell maculae. Further studies on the relationship between sagitta size, saccular macula hair cell number and density, and auditory physiology in serrasalmids would be necessary to determine (a) if otolith size is correlated with hair cell number; (b) if smaller otoliths are associated with lower auditory thresholds in noisy environments; and (c) if smaller otoliths are able to displace a comparable number of hair cell kinocilia at a given sound intensity. Modeling of otoliths that are not involved in indirect sound pressure detection (i.e., otoliths in nonotophysan fishes) suggests that greater otolith masses should be tuned to lower frequencies and have a narrower response range (Lychakov & Rebane, 2000; Lychakov & Rebane, 2002). Thus, the relatively low lapillus (utricular otolith) and asteriscus (lagenar otolith) masses of rheophilic serrasalmids may have a broader overall response and be tuned to higher frequencies. Smaller otoliths in rheophilic serrasalmids could be an adaptation to aid in higher frequency hearing, perhaps in response to increased ambient noise at low frequencies. Notably, in the nonotophysan teleost family Ambylopsidae, cave-dwelling species, which live in subterranean stream environments that are noisier from 1 to 3 kHz than the stream and pool environments of their surface-dwelling relatives, have lower hair cell densities and cannot hear as high of frequencies as surface living species (Niemiller, Higgs, & Soares, 2013). Thus, an alternative hypothesis is that smaller otoliths of rheophilic serrasalmids could be a reduction in investment of hearing-related morphological features in response to life in an overall noisier habitat. Audiometric studies in these species and in situ noise measurements in habitats occupied by rheophilic serrasalmids are necessary to evaluate these hypotheses further. The utriculus is thought to function primarily in detection of linear accelerations as part of the

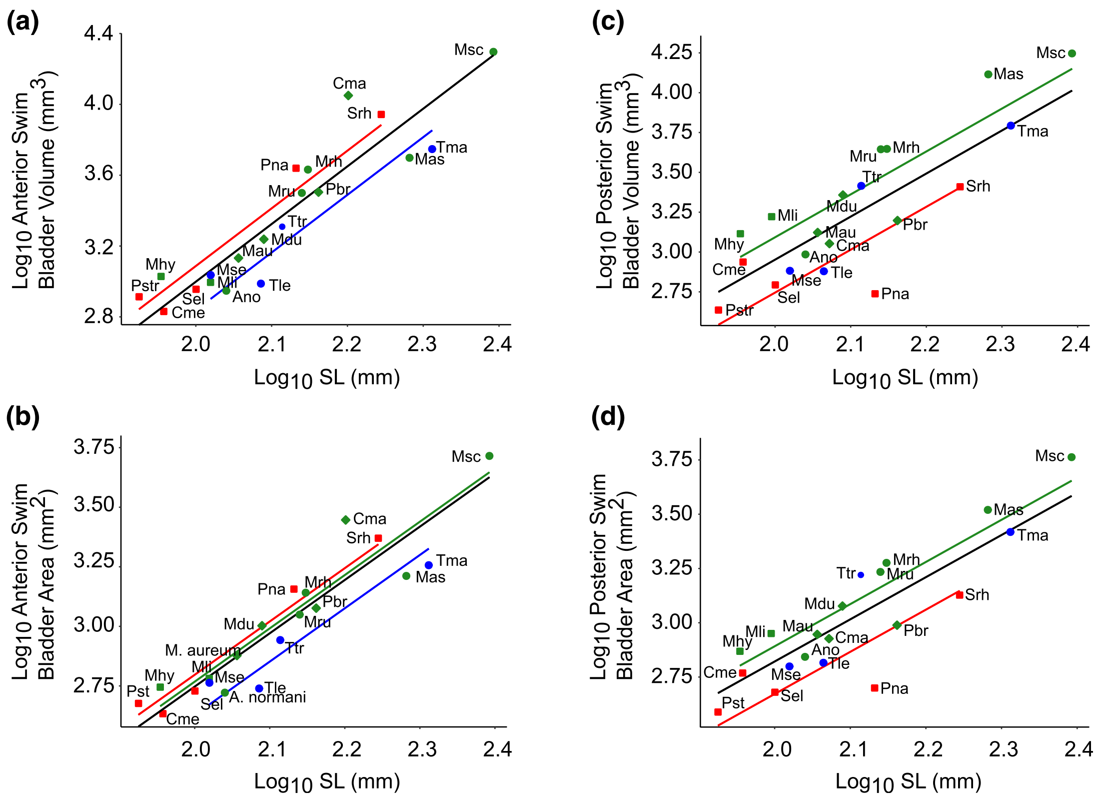

FIGURE 6 Relative size relationships of the anterior and posterior swim bladder chambers among serrasalmid species. (a) Anterior swim bladder volume vs. standard length (SL), (b) anterior swim bladder surface area vs. SL, (c) posterior swim bladder volume vs. standard length (SL), and (b) posterior swim bladder surface area vs. SL. Size data were $\log_{10}$ transformed. Fitted lines determined from maximum likelihood λ PGLS models (Tables 8 and 9). Black lines are fits among all species. Colored lines present when significant differences (Tables 8 and 9) were observed among some or all ecological groups: Green = frugivore/carnivores, red = carnivores, blue = rheophiles

vestibular sense (Ladich & Schulz-Mirbach, 2016). Models also suggest that smaller otoliths are less sensitive to vestibular stimuli, have lower resolving power, a broader operational range of acceleration stimuli, and a faster response than large otoliths (Lychakov & Rebane, 2000). These features may be important for balance and movement in high-energy environments.

4.2 | Frugivore/granivores compared to carnivores

A frugivore/granivore mode of life was most likely the ancestral state of serrasalmids and among the taxa in this study, carnivory likely evolved only within the piranha clade (Figure 1). In general, frugivore/granivores and carnivores in this study displayed modest differences in auditory structure size. Frugivore/granivores had larger sagittae and larger asterisci in ML models than carnivores. As discussed above, sagittae in otophysans are involved in the indirect detection of sound pressure via transduced fluid flow, and larger sagittae, because of their increased mass and inertia may require more endolymphatic flow to produce displacements sufficient to stimulate hair cells. However, larger saggitae may have the potential to trigger more kinocilia of hair cell maculae, assuming an increase in size is accompanied by a larger sulcus and corresponding macula. In otophysans, asterisci are likely to be involved in detection of sound particle motion in addition to their role in the vestibular sense (Popper & Fay, 2011). Larger asterisci are predicted to have a sharper response to low frequencies and a greater delay to response than smaller asterisci (Lychakov & Rebane, 2000). The large sizes of the frugivore/granivore asterisci suggest that particle motion detection may be enhanced in many of these species, which is consistent with the prediction that such stimuli may be important when fruits and seeds produce sounds when falling into rivers. Further, the larger asteriscus size of frugivore/granivores and carnivores compared to rheophiles indicates that particle motion detection may be more important in these two groups and potentially made possible by the quieter environments occupied by these species.

4.3 | *Acnodon normani*

Acnodon normani has a relatively unique ecological position among the species in this study. The reported trophic niche breadth of this species appears broad and includes, not only fruits and seeds, but other plant material, invertebrates, and fish scales (Leite & Jégu, 1990). Further, this species is relatively elongate in body morphology, especially in comparison with other *Myleus*-clade members and has large orbits and eyes. *Acnodon normani* has also been reported from rapids, co-occurring with *M. setiger* and *Tometes* spp. (Andrade, Sousa, Ota, Jégu, & Giarrizzo, 2016) and fast flowing streams (Crampton, 2011), but is also known from habitats with more moderate flow (Pereira, Agostinho, de Oliveira, & Marques, 2007). This species differed from most other frugivore/granivores in this study in having smaller relative

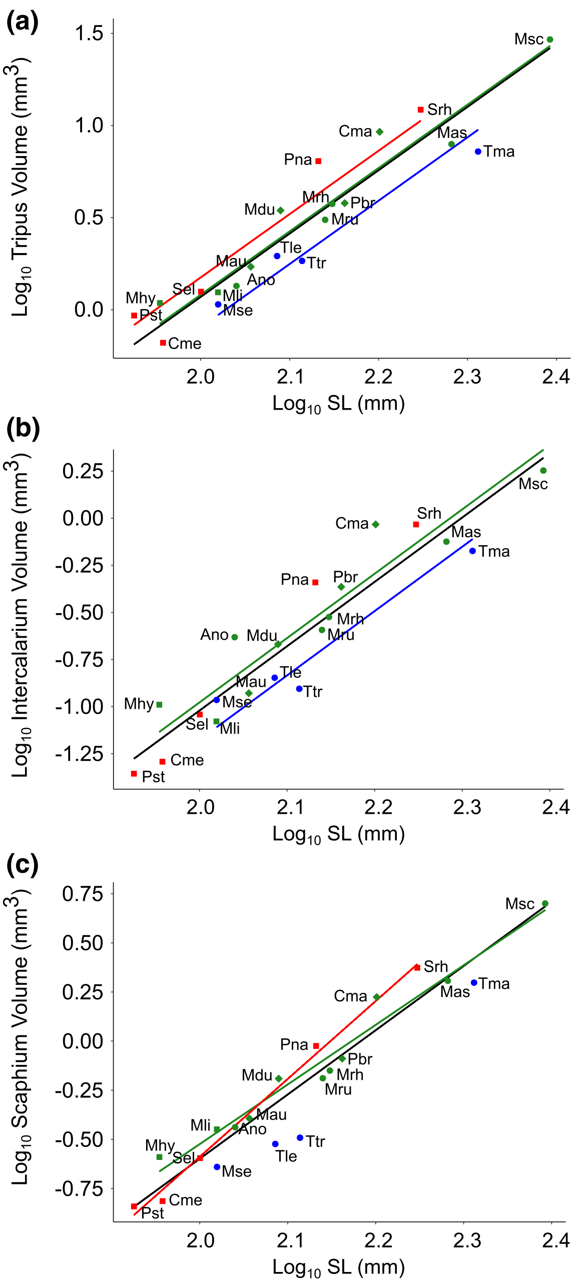

FIGURE 7 Relative Weberian ossicle size relationships among serrasalmid species. (a) Tripus volume vs. standard length (SL), (b) intercalarium volume vs. SL, and (c) scaphium volume vs. SL. Size data were log$_{10}$ transformed. Fitted lines determined from maximum likelihood λ PGLS models (Table 2). Black lines are fits among all species. Colored lines present when significant differences (Table 10) were observed among some or all ecological groups: Green = frugivore/carnivores, red = carnivores, blue = rheophiles

sizes of the tripus, sagitta, and lapillus, in accordance with the trend observed in rheophiles. However, *A. normani* was distinct from rheophiles in having a relatively large intercalarium, scaphium, and asteriscus, like other frugivore/granivores. The anterior swim bladder chamber of *A. normani* is relatively small, while the relative size of the posterior swim bladder chamber is near the average among serrasalmids examined. Swim bladder size in *A. normani* is likely affected in part by the relatively slender body morphology of this species, in which a large total swim bladder volume would provide too much lift.

4.4 | Otolith size

Otolith size among species may be influenced by the importance of sound production in communication. Large otoliths have been demonstrated to occur among soniferous fish species (Cruz & Lombarte, 2004; Paxton, 2000). Sound production has been documented for several carnivorous piranha species (Ladich, 1999; Markl, 1971; Mélotte et al., 2016; Millot et al., 2011; Stabentheiner, 1988). Their low-frequency drumming sounds have the potential to directly stimulate the lagena in the acoustic near field, which extends further at low frequencies (Mann, 2006). Thus, selection pressures for both larger asterisci and sagittae, for detecting particle motion and sound pressure components of vocalizations, respectively, may exist for serrasalmids that may produce sounds for communication.

4.5 | Swim bladder size and morphology

The size of the swim bladder chambers in these fishes is likely to be influenced by a combination of factors: its role in sound pressure detection with Weberian ossicles, its role as a sonic organ, and its role in buoyancy regulation. Smaller anterior swim bladder chamber sizes of rheophilic species could be a response to increased lift from water flowing over the body and fins in a higher current environment. Overall, anterior swim bladder sizes among frugivore/granivores and carnivores were not distinctive. Among at least the piranhas (carnivores of the piranha clade), the anterior swim bladder chamber, in addition to the role of pressure receptivity for the Weberian apparatus, is part of a sonic organ driven by vibrations of extrinsic sonic muscles (Mélotte et al., 2016). In some of the species, for example, *Pygocentrus nattereri* and *Serrasalmus rhombeus*, the relative size of the anterior chamber is quite large. While no differences were observed between frugivore/granivores and carnivores, the posterior chamber of frugivore/granivores was found to be larger in ML models. Evolutionary changes to the size of the anterior chamber are likely to be inversely related to size changes in the posterior chamber to maintain the size of the swim bladder and thus the overall role in buoyancy. In this study, several unusual features of swim bladder morphology were observed. The caudal tips of the posterior swim bladder chambers of *Mylossoma* spp. (Figure 2d) and *Serrasalmus* spp. (Figure 3e) observed in this study extended beyond the precaudal vertebrae and continued to the right or left (depending on the specimen) side of the haemal spines, a condition which has been reported previously (Nelson, 1961). In addition, the anterior chamber of *Mylossoma* spp. (Figure 2d) contains diverticulae (Nelson, 1961) that extend anteriorly, below the neurocranium, as well as posteriorly, lateral to the anterior portion of the anterior chamber and medial to the cleithrum. It is not known if these narrow diverticulae serve an auditory role. Some of the extensions extend relatively close to the thin lagenar bullae (formed by the basioccipital and exoccipital), and some continue below the prootic (Figure 2d). Serrasalmids, like other characiform fishes, have a foramen in the prootic (Figure 2a,d) that is open to the utricule (Fink & Fink, 1981). Some of the diverticulae extend toward the prootic foramen, but most are not oriented toward the foramen. There is experimental evidence from fish audiometry studies indicating that in species that lack an otophysic connection, swim bladder proximity to the inner ears can

TABLE 10 Results from PGLS and maximum likelihood (ML) tests of λ for models that investigated covariation between Weberian ossicle sizes (volume) and body length for 20 serrasalmid species

Model, ML test Model terms	d.f.	*F*-stat	Est.	SE	*t*-val.	*p*	r^2 adj	Log likelihood	AICc
Tripus volume									
λ 95% CI: 0.00–0.61									
λ = 0? *p* = .650									
λ = 1? *p* < **.001**									
ML λ = 0.00	3,16	79.0				**<.001**	0.925	16.17	−24.35
Intercept			−6.70	0.474	−14.15	**<.001**			
SL			3.44	0.229	15.00	**<.001**			
f vs. c			−0.09	0.068	−1.38	.186			
r vs. c			−0.27	0.083	−3.27	**.005**			
r vs. f			−0.18	0.070	−2.52	**.023**			
Intercalarium volume									
λ 95% CI: 0.00–0.73									
λ = 0? *p* = 1.00									
λ = 1? *p* = **.012**									
ML λ = 0.00	3,16	54.2				**<.001**	0.894	12.06	−16.12
Intercept			−7.82	0.582	−13.44	**<.001**			
SL			3.42	0.282	12.13	**<.001**			
f vs. c			0.01	0.083	0.17	.864			
r vs. c			−0.18	0.102	−1.81	.090			
r vs. f			−0.20	0.086	−2.29	**.036**			
Scaphium volume									
λ 95% CI: 0.00–0.89									
λ = 0? *p* = 1.00									
λ = 1? *p* = **.033**									
ML λ = 0.00	5,14	131.0				**<.001**	0.972	27.74	−43.48
Intercept			−8.51	0.553	−15.40	**<.001**			
SL			3.96	0.269	14.74	**<.001**			
f vs. c			1.92	0.677	2.84	**.013**			
r vs. c			0.99	0.900	1.11	.289			
r vs. f			−0.93	0.809	−1.15	.270			
SL*f vs. c			−0.93	0.325	2.86	**.013**			
SL*r vs. c			0.60	0.427	−1.40	.185			
SL*r vs. f			0.30	0.378	0.88	.394			

Abbreviations: c = carnivores, Est. = estimate, f = frugivore / granivores, r = rheophilic, SL = standard length. For comparisons among ecological groups, coefficient estimates indicate the magnitude and relative relationship of the groups (e.g., f vs. c, when positive indicates frugivores are relatively higher for that size trait than carnivores).

increase sensitivity toward sound pressure (Parmentier, Mann, & Mann, 2011; Tricas & Boyle, 2015; Yan et al., 2000). However, it is not clear what possible additional auditory function such diverticulae would provide for a fish with a well-developed Weberian apparatus. Similar diverticulae are present in some doradid catfishes, but a clear functional role in hearing has not been observed (Zebedin & Ladich, 2013).

4.6 | Relationships with pressure and particle motion sensitivity

A recent study on hearing capacities in serrasalmid fishes, indicated that broad overlap exists in the frequency range of hearing among species (Mélotte et al., 2018). Further, audiogram variation was not clearly associated with feeding ecology or with vocal ability (Mélotte et al., 2018). In the Mélotte et al. (2018) study, *P. brachypomus*, a member of the basal pacu-clade was most divergent along many mid-range frequencies 900–1,500 Hz, while members of the *Myleus*-pacus and piranha clades were largely similar. Auditory ranges from other members of the basal pacu-clade, however, are not yet known. In the Mélotte et al. (2018) study, while carnivore and frugivore audiograms largely overlapped, the greatest separation occurred in lower frequencies 50–1,200 Hz, where carnivores tended to have lower thresholds. Hearing sensitivity in carnivorous serrasalmids may be important for detecting splash sounds. Splash sounds at the surface of the water are attractive to carnivorous piranhas (Markl, 1972). Splashing sounds of fishes can be broad-band and contain relatively high frequency components (up to several kHz) (Bolgan et al., 2016; Phillips, 1989). At

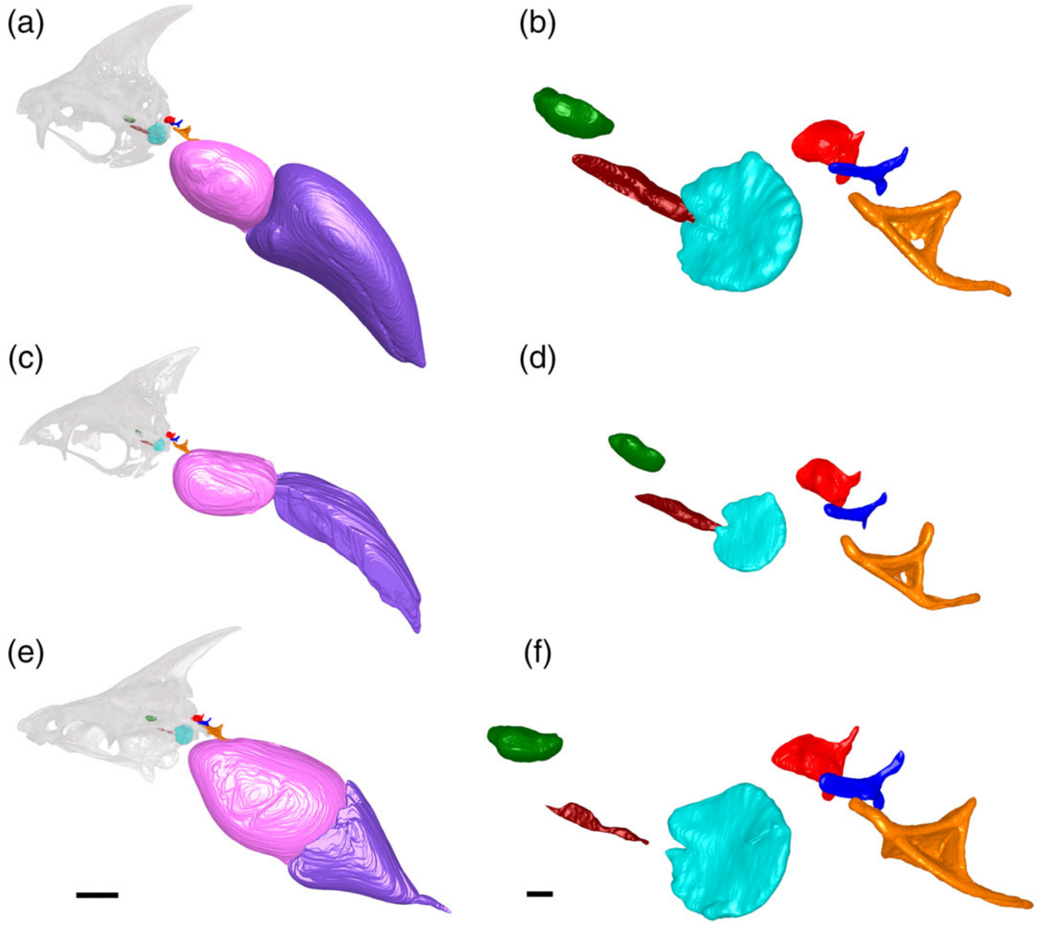

FIGURE 8 Surface reconstructions of the neurocranium, vertebrae 1–4, neural complex, Weberian ossicles, and swim bladder of comparably sized serrasalmid specimens of three broad ecological groups: (a, b) *Myleus asterias* (MNHN IC.1997.151, 185 mm SL), a frugivore/granivore; (c, d) *Tometes makue* (MNHN IC.2001–2,708, 178 mm SL), a rheophile; and (e, f) *Serrasalmus rhombeus* (MNHN IC.2007–0901, 175 mm SL) a carnivore. (a, c, e) left lateral views of the neurocranium, vertebrae 1–4, Weberian ossicles, otoliths, and swim bladder chambers. The neurocranium was rendered transparent to show the position of the otoliths. (b, d, f) left lateral views of the Weberian ossicles (scaphium, intercalarium, tripus) and otoliths (utricular otolith = lapillus, saccular otolith = sagitta, lagenar otolith = lapillus). Note the relative size differences of the swim bladder chambers, Weberian ossicles, and otoliths of comparably sized fishes that are shown at the same scale

TABLE 11 Summary of ecological group differences in size and scaling relative to body size of otoliths, swim bladder chambers, and Weberian ossicles among serrasalmids

Structure and feature	Size relationships among ecological groups			Phy. Signal
Otoliths				
Sagitta length		Ns		Strong
Sagitta volume	Frug./gran.	>	Carn. + rheoph.	Weak
Sagitta surface area	Frug./gran.	>	Carn. + rheoph.	Moderate
Asteriscus volume	Carn.	> *scaling*	Frug./gran.	Weak
Asteriscus surface area	Carn.	>	Rheoph.	Weak
Lapillus volume	Carn.	> *scaling*	Frug./gran. + rheoph	Weak
Lapillus surface area	Carn.	> *scaling*	Rheoph.	Weak
Swim bladder				
Anterior chamber volume	Carn.	>	Rheoph.	Weak
Anterior chamber area	Carn. + frug./gran.	>	Rheoph.	Weak
Posterior chamber volume	Frug./gran.	>	Carn.	Moderate
Posterior chamber area	Frug./gran.	>	Carn.	Weak
Weberian ossicles				
Tripus volume	Carn. + frug./gran.	>	Rheoph.	Weak
Intercalarium volume	Frug./gran.	>	Rheoph.	Weak
Scaphium volume	Carn.	> *scaling*	Frug./gran.	Weak

Abbreviations: carn. = carnivores, frug./gran. = frugivore / granivores, Phy. = phylogenetic, r = rheophilic.

higher frequencies (>1,200 Hz), however, there is broad overlap and variability among frugivore/granivores and carnivores (Mélotte et al., 2018) and, as mentioned above, splash sounds, which may be more important for carnivores, likely include these higher frequency components. In addition, sensitive hearing may be important for detecting communication sounds and low frequency vocalizations are known from more carnivore species in the piranha clade than other serrasalmids (Mélotte et al., 2016). It is not yet known if rheophiles diverge in auditory characteristics from the species in the Mélotte et al. (2018) study. In addition, the relationship between sound pressure and particle motion detection remain largely unknown, because of the challenges of testing these two aspects of sound stimuli independently in a laboratory (Popper & Fay, 2011). These two aspects of sound have frequency dependent characteristics that vary with source distance (Mann, 2006). It remains to be determined if differences in auditory morphology among ecological groups are associated with differences in how these taxa detect relevant sound cues. Moreover, it remains unknown if differences among particle motion sensitivity exist among serrasalmids. Differential input from sound pressure and particle motion may be especially important for how fishes localize a sound source (Fay, 2011).

5 | CONCLUSIONS

This study demonstrated that in Serrasalmidae, size-related features of Weberian ossicles, swim bladder chambers, and otoliths, are influenced to varying degrees by ecology and phylogenetic history. Rheophiles appear to show the most divergence from other serrasalmids in the relative sizes of individual elements of the Weberian apparatus and otoliths, with reductions in size of the sagitta, asteriscus, tripus, and intercalarium. Frugivore/granivores show differences from other serrasalmids in having the largest sagittae. Several of the morphological features examined in this study have multiple known functions and are likely influenced by multiple selection pressures. The swim bladder has a role in buoyancy, sound reception, and sound production. The otoliths are involved in detection of sound particle motion, indirect sound pressure reception (mainly sagittae), and the vestibular sense. A hypothesized fundamental role of hearing in vertebrates is analysis of the auditory scene (Popper & Fay, 1993, Fay & Popper, 2000, Popper et al., 2003). How auditory scene analysis is used by serrasalmids with different ecologies may be a selective force that has influenced differences in the relative sizes of Weberian ossicles and otoliths observed in this study. A rheophilic ecology in serrasalmids is a derived condition that most likely evolved from an ancestral ecology of frugivory in slow-moving waters. The size-related differences in Weberian ossicles and otoliths observed in rheophiles may be associated with the influence of noise. Notably, quiet, shallow waters are hypothesized to have played a role in the evolution of higher frequency hearing in otophysans (Ladich & Popper, 2004; Popper et al., 2003; Rogers & Cox, 1988). Perhaps the reduction in size of otoliths and Weberian ossicles of rheophilic serrasalmids could help retain higher frequency responses in the presence of low frequency background noise or, alternatively, could be a reduction in investment of structures that are less effective for enhanced hearing in a noisy environment. Future studies on serrasalmids are warranted to (a) characterize the acoustic environments of species with different ecologies, (b) determine how sound influences the behavior (feeding and social behavior), and (c) to determine the auditory capacities associated with different otolith and accessory auditory structure morphologies.

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