Zoological Journal of the Linnean Society, 2024, **202**, zlae135
https://doi.org/10.1093/zoolinnean/zlae135
Advance access publication 6 November 2024
Original Article

Original Article

Adaptation and innovation in darter fish cranial musculature (Etheostomatinae: Percidae): insights from diceCT

J.H. Arbour[1,*], S. Ramazan[1], S. Clark[1]

[1]Department of Biology, Middle Tennessee State University, Murfreesboro, TN, USA

[*]Corresponding author. Department of Biology, Middle Tennessee State University, 1301 E Main Street, Murfreesboro, TN, 37132, Box 60, USA. E-mail: jessica.arbour@mtsu.edu

ABSTRACT

Fish skulls are often highly kinetic, with multiple linkage and lever systems powered by a diverse suite of muscles. Comparative analysis of the evolution of soft-tissue structures in the fish skull is often limited under traditional approaches, while new imaging techniques like diceCT (diffusible iodine-based contrast-enhanced computed tomography) allow for high-resolution imaging of muscles *in situ*. Darters (Percidae: Etheostomatinae) are a diminutive and species-rich clade of lotic freshwater fishes, which show diverse head shapes believed to be associated with different foraging strategies. We used diceCT to sample all major cranial adductors and abductors responsible for movement of the jaw, hyoid, operculum, and suspensorium from 29 species. We applied comparative phylogenetic approaches to analyse the evolutionary trends in muscle size across the clade. We found two major patterns: (i) darter cranial muscles show fundamental trade-offs relating to investment in musculature, as well as buccal expansion vs. biting attributes; early divergence in muscle size appears to be associated with shifts in habitat use and foraging; (ii) darter adductor mandibulae show high variation in architecture (fibre orientation, divisions). This study highlights how new imaging techniques can provide novel insights into the anatomy of even well-sampled/represented clades.

Keywords: functional morphology; anatomy; evolution; perciformes; adductor mandibulae; phylogenetic comparative methods

INTRODUCTION

The skulls of fishes are frequently highly kinematic, with multiple muscle groups responsible for dorsal, ventral, lateral, and medial movements of the oral jaws, hyoid, suspensorium, and opercular series (Westneat 1990, 1994, Muller 1996, Camp and Brainerd 2015, Camp *et al.* 2015). This kinematic complexity has allowed different species of fish to diversify across the three major feeding modes: ram feeding—overtaking and engulfing prey; suction feeding—drawing prey through a suction gradient; and biting—grasping and directly manipulating prey. Ram feeding requires quick expansion of the oral jaws, and is often associated with rapid oral jaw kinematics and large gapes, while suction feeding is benefitted by fast and considerable expansion of the buccal cavity and small gapes (Wainwright and Richard 1995, Wainwright *et al.* 2001, Carroll and Wainwright 2009, Hulsey *et al.* 2010). Fishes relying primarily on biting/manipulation behaviours are often associated with force-optimized kinematics and large adductor mandibulae (Turingan 1994, Kolmann *et al.* 2014).

Variation in muscle anatomy impacts performance, such as through force production or speed. Muscle size is generally a major predictor of the force production capability of a particular muscle and is often characterized via the physiological cross-section area (PCSA), which a strong predictor of force output and is proportional to muscle volume (i.e. measured via mass X density in many studies) divided by fibre length. These factors (mass/volume, fibre length) may have variable impacts on performance. Herrel *et al.* (2008) found that in bats (a vertebrate group with highly variable skull shapes and musculature), a model incorporating only muscle mass explained 63% of variation in measured bite force, and fibre length improves this by only an additional 13%. Muscles are also energetically expensive, and muscle size may be reflective of functional demands/priority, especially when space constraints are limiting (Hulsey *et al.* 2007, O'Brien *et al.* 2019, Somjee 2021). Differential investment in muscles associated with expansion of the jaws and buccal cavity (e.g. the levator arcus palatini and dilatator opercula), compared with those associated with biting (adductor mandibulae) or rapid opening of the oral jaws (protractor hyoidei and sternohyoideus) may illustrate relative investment in suction, biting, and ram-feeding modes (Wainwright *et al.* 2004, López-Fernández *et al.* 2013). Muscle force output is maximized

Received 6 March 2024; revised 23 September 2024; accepted 1 October 2024

with shorter muscle fibres, while fibre length is positively correlated with muscle contractile speed (Gans and de Vree 1987), thus producing a trade-off between force and velocity production in muscle architecture. Shorter fibres increasing overall force output may be driven from changes in muscle morphology (shorter muscle length), or through changes in pennation. Pennate muscles have fibres attached obliquely to the central tendon (either to one side of the tendon—unipennate—or on both sides—bipennate), in contrast with parallel or fusiform muscles, which have fibres running parallel to the long axis of the muscle. This reduces muscle fibre length for the same overall volume in pennate muscles (i.e. increasing force; Martin *et al.* 2020), but reduces range of motion.

Contrast-enhanced computed tomography techniques like diceCT provide an opportunity to examine the evolution of soft-tissue structures (e.g. muscles) in groups not previously accessible to traditional dissection techniques; such as those with rare specimens (extinct, of conservation concern, or difficult to access) or very small animals (Baverstock *et al.* 2013, Gignac *et al.* 2016, Gignac and Kley 2018, Brocklehurst *et al.* 2019, Kolmann *et al.* 2023). Myological features such as muscle volume, origins/insertions, muscle fibre length/curvature, and pennation angles can all be sampled using these techniques, with significantly less permanent damage to specimens, i.e. chemical rather than 'physical' (Dickinson *et al.* 2018, Santana 2018, Sullivan *et al.* 2019, Katzke *et al.* 2022). Typical applications of diceCT approaches have involved the digital dissection of a small number of individuals for anatomical descriptions (Dickinson *et al.* 2019, Sullivan *et al.* 2019, Cox *et al.* 2020). Some recent studies have used this approach in a macroevolutionary context to study the origin of trait diversity and ecological adaptations, such as examining brain structure in squamates (Macrì *et al.* 2023), venom glands in snakes (de Oliveira *et al.* 2024), and cranial muscles in bats (Santana 2018), among others. Fish functional morphological studies stand to benefit greatly from such imaging and digital dissection approaches (Kolmann *et al.* 2023), but few studies thus far have applied diceCT approaches in comparing investment in different muscle groups in a macroevolutionary/comparative context in fishes.

In this study we provide the first myological account of the major cranial adductor and abductors in a diverse radiation of freshwater fishes, the darters (Etheostomatinae: Percidae). With more than 200 species found across North America, these fish species are generally found in rocky, fast-flowing waters, but with some species living in sandy or calm habitats, and typically in close association with the benthos. While largely benthic invertivores feeding on aquatic insects (e.g. chironomid and Ephemeroptera larvae), other invertebrates, and algae/detritus, they employ divergent foraging strategies (Turner 1921, Paine *et al.* 1982, Orr 1989, Carlson and Wainwright 2010) and show a diversity of head shapes (Fig. 1). Carlson and Wainwright (2010) identified three major, specialized ecomorphs based on skeletal morphology and observation of feeding behaviours: (i) the rock flippers, who use long, conical snouts to overturn rocks to search for prey (Fig. 1; *Percina caprodes* Rafinesque, 1818); (ii) probers that insert long, narrow snouts into crevices (Fig. 1; *Percina squamata* Gilbert and Swain, 1887); and (iii) 'manipulators' with shortened, downturned faces that pry prey from the upper surfaces of rocks or from the exposed substrate between rocks (Fig. 1; *Etheostoma blennioides* Rafinesque, 1819, *E. barrenense* Burr and Page, 1982, and *E. blennius* Gilbert and Swain, 1887). Carlson and Wainwright (2010), suggest that biting and suction are probably major feeding modes employed by darters. They note that species in *Ammocrypta*, *Crystallaria*, and *Percina* with low jaw-closing ratios probably employ suction feeding to forage in soft, sandy substrates. Orr (1989) observe *Nothonotus jordani* (Gilbert, 1891) aggressively tearing apart large prey, and species like *Etheostoma flabellare* (Rafinesque, 1819) are frequently documented with stomach contents containing very large prey from beneath and between rock crevices (Turner 1921, Paine *et al.* 1982), and are likely to be more benefitted by gripping and biting. Previous quantitative analyses of darter functional morphology have focused on skeletal features through histological 'cleared and stained' specimens (Carlson and Wainwright 2010). No prior studies have undergone a quantitative comparison of the relative contributions of the various cranial muscles, nor how these may relate to trade-offs between the various documented foraging modes. This is partially due to the diminutive size of these species (ranging from 4.3 to 20 cm total length), the difficulty of manual dissection of the smaller muscles (e.g. opercular adductors and levators), and the rarity/conservation status of several species (some even critically endangered or declared extinct). Anatomical descriptions are available only for the other subfamilies of the Percidae—*Perca* and *Gymnocephalus* from Percinae (Osse 1969 *et al.* 1976), and *Sander* from Luciopercinae (Elshoud-Oldenhave 1979), some of which are in fairly inaccessible monographs (due to rarity of prints in library collections). The purpose of this study was to (i) provide a description of the anatomy and variation of the major adductors and abductors associated with the jaw, suspensorium, and opercular series in darters, and (ii) determine the major evolutionary trends in relative muscle investment across these systems in the darter subfamily. We used diceCT, digital dissection, and phylogenetic comparative approaches to quantify darter cranial musculature across 29 species, which revealed innovation in biting muscle architecture and signals of early adaptive evolution associated with fast-rocky vs. slow-sandy river habitat trade-offs.

MATERIALS AND METHODS

CT scanning

We digitized the skull and major cranial muscles from 29 species, representing the genera *Ammocrypta* ($N = 1$), *Crystallaria* ($N = 1$), *Etheostoma* ($N = 18$), *Nothonotus* ($N = 3$), and *Percina* ($N = 6$). Specimens were obtained from natural history collections (University of Michigan - UMMZ, Yale Peabody Museum - YPM-ICH and the MTSU zoological collection, and see Table S1) and through field sampling (MTSU IACUC protocol #22-3001). Field-captured specimens were fixed in 10% neutral buffered formalin prior to storage in 70% ethanol. Specimens were scanned on a Scano Medical uCT50 scanner in the Vanderbilt Small Animal Imaging Lab at the Vanderbilt Institute for Imaging Sciences. An initial unstained scan was taken for skeletal reconstruction and specimens were then stained for 1–4 days in 1% Lugol's iodine (1% I_2 by weight/volume). Specimens were soaked in distilled water for 30 min to 1 h prior to the

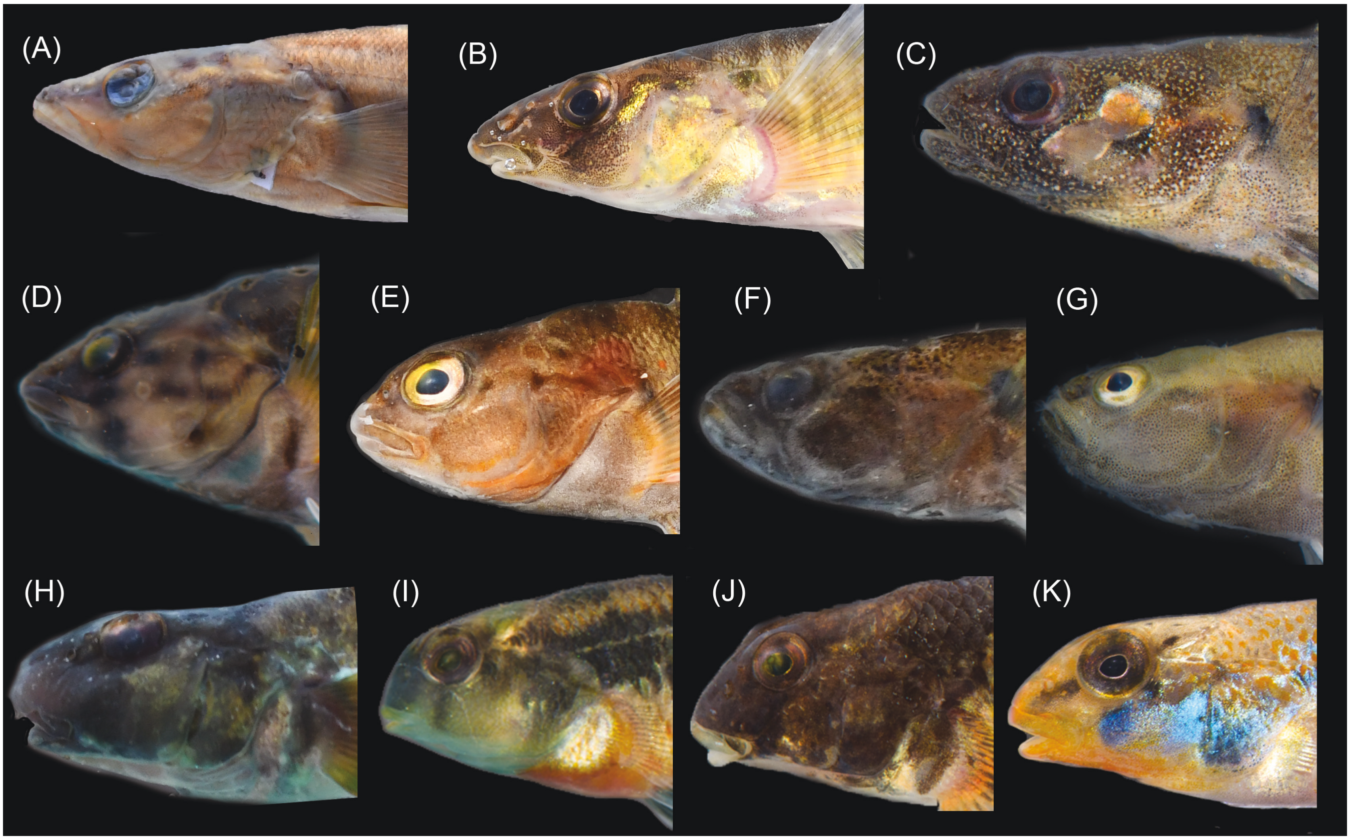

Figure 1. Diversity of head shapes in darters, showing variation in the elongation of the head, length of the snout, position of the mouth and eyes and size of the mouth, among other features. Species shown: A, *Percina squamata*; B, *Percina caprodes*; C, *Etheostoma derivativum*; D, *Nothonotus rufilineatus*; E, *Nothonotus camurus*; F, *Etheostoma flabellare*; G, *Etheostoma kennicotti*; H, *Etheostoma blennioides*; I, *Etheostoma barrenense*; J, *Etheostoma blennius*; K, *Etheostoma jimmycarter*. Image credits to: J. Arbour and N. Schroth.

second stained scan, to minimize gradients in staining and to improve contrast. Specimens were wrapped in a combination of cheesecloth (unstained only), plastic shipping wrap, and parafilm for scanning. All scans were conducted using the following parameters: 10–17.2 μm, 55 kV,200 uA, and a 0.5 mm Al filter. Radiographs were reconstructed into TIFF image stacks using the Scanco proprietary reconstruction software.

Digital dissections

Reconstruction of the cranial skeleton and musculature was conducted in 'MIMICS' v.25 (Materialise). Skeletal reconstructions primarily used the thresholding tools, while muscles were manually segmented using the 'multiple slice edit' tool. Muscle boundaries were outlined across multiple locations and the mask on the remaining slices was interpolated. The following muscles were digitally dissected from the right side of each specimen: the adductor mandibulae (AM), segmentum fascialis, pars malaris (traditionally AM1), rictalis (AM2), and stegalis (AM3) where visible, the adductor arcus palatini, the levator arcus palatini, the dilatator operculi, the adductor operculi, the levator operculi, the adductor hyomandibulae, the geniohyoideus/protractor hyoidei, and the sternohyoideus. These represent the largest cranial muscles related to the opening and closing of the oral jaws, buccal cavity, and hyoid apparatus. Their functions are described individually in Table 1. The CT and diceCT scans were aligned using a roughly thresholded model of the diceCT scan and N-point registration in Geomagic, using visible skeletal points in the diceCT full head/skull model to overlap the models.

In most major teleost fish clades, the pars malaris and pars rictalis are superficial, with malaris dorsal to rictalis; the smaller stegalis is deep and largely or entirely covered by the two other divisions of the AM (Datovo and Vari 2013). We followed Datovo and Vari (2014) for the nomenclature of the AM muscle complex, including for combinations of the three divisions (e.g. perch and walleye possess a combined AM pars rictalis and AM pars stegalis, henceforth referred to as the rictostegalis). Where significant differences in fibre arrangements occurred across each division, or clear additional subdivisions of the AM could be seen, divisions were segmented separately and nomenclature follows Datovo and Vari (2014).

Comparative analysis of darter cranial musculature

We tested whether darter species show differential investment into the size of muscles associated with various cranial functions (abduction/adduction of the oral jaws, buccal cavity, and opercular series). We calculated the volume of each muscle in Geomagic Wrap. While muscle shrinkage is a possible side-effect of staining with Lugol's iodine (Brocklehurst *et al.* 2019), we note that all specimens were already preserved in ethanol, which on its own will dehydrate specimens, and thus measures will be an underestimate of total muscle volume in fresh specimens. Herein we assume that shrinkage will be generally consistent across the

Table 1. Muscle groups dissected from each of 29 species of darters, including nomenclature of their major divisions as appropriate and their primary action/function.

Muscle	Function
Adductor mandibulae (malaris, stegalis and rictalis or rictostegalis where applicable)	Closing of the oral jaws/adduction of the lower jaw
Adductor arcus palatini	Medial retraction of the suspensorium (decrease buccal volume)
Levator arcus palatini	Lateral expansion of the suspensorium (increase buccal volume)
Dilatator operculi	Rotation and abduction of the operculum (increase opercular cavity volume)
Adductor operculi	Retraction of the operculum (decrease opercular cavity volume)
Adductor hyomandibulae	Retraction of the suspensorium (hyomandibula)
Levator operculi	Lateral abduction of the operculum (increase opercular cavity volume)
Geniohyoideus/protractor hyoidei	Adduction of the hyoid bar (when mouth is closing)
Sternohyoideus	Depression of the hyoid bar (expansion of the buccal cavity). Indirectly depression of the lower jaw (opening of the oral jaws)

various species, although it is possible that shrinkage could vary with specimen preservation technique, age, or other factors. Since our goal in the following analyses is to quantify interspecific patterns rather than provide absolute estimates of bite force, we believe any shrinkage will not overly bias our overall results.

We used phylogenetic comparative approaches to examine the overall evolutionary pattern of investment in cranial musculature across the darter subfamily. The total volume of each muscle was size-corrected using a log-log regression on body size (standard length—SL). These size corrections were carried out with phylogenetic correction using the R function 'phyl.resid' from the package 'phytools' (Revell 2009, 2012). We used a subtree of the phylogeny from Arbour and Stanchak (2021) for this and subsequent macroevolutionary analyses. We used phylogenetically corrected principal component analysis (pPCA) on the size-corrected muscle volumes to determine the major axes of variation in darter cranial musculature, using the function 'phyl.pca' from the R package 'phytools' (Revell 2009, 2012). We tested for evidence of selection/adaptive evolution using shifts from a constant rate, random walk process (e.g. Brownian motion, BM) using two approaches. We used disparity through time (DTT) analysis to quantify how muscle-size disparity has been partitioned in the evolution of darters. We quantified subclade disparity for each PC axis at each node in the phylogeny and contrasted this with 100 simulations from a BM process using the morphological disparity index (MDI) following Slater *et al.* (2010a), Slater and Pennell (2014). DTT tests were carried out using functions from the R package 'GEIGER' (Harmon *et al.* 2008). We excluded the final 25% of the tree depth (time) to account for incomplete taxonomic sampling when calculating the MDI value, using the argument 'mdi.range' in the function 'dtt' (Harmon *et al.* 2003, Slater *et al.* 2010b). Negative MDI values are typical of early bursts of trait evolution or divergent selection across multiple adaptive peaks, while positive MDI values may be indicative of either accelerating rates of evolution or strong selection towards a single selective optimum (López-Fernández *et al.* 2013, Slater and Pennell 2014, Arbour and López-Fernández 2016).

We used 'l1ou' to detect shifts in selection across muscle size in darters (Khabbazian *et al.* 2016). This approach uses Ornstein–Uhlenbeck models, which extend BM models by incorporating selection towards one or more adaptive peaks. The R package 'l1ou' uses a lasso approach to detect shifts to new adaptive peaks without an *a priori* hypothesis. We detected shifts in adaptive peaks using 'estimate_shift_configuration' and convergence between shifts using 'estimate_convergent_regimes', and evaluated support using AICc (sample size corrected Akaike Information Criterion) values. We found that other information criteria (e.g. pBIC, phylogenetic Bayesian Information Criterion) produced more numerous and more poorly supported shifts, but always produced those found by AICc, which was the most conservative metric tested. Bootstrap support for adaptive peak shifts was determined using 'l1ou_bootstrap_support' (100 iterations).

RESULTS

Anatomical descriptions of darter cranial musculature

Here we detail the major patterns of insertion, attachment, pennation, and major muscle divisions for the muscles outlined in Table 1. We note those features that were variable across the darters examined, and those features that differed from prior anatomical accounts of *Perca* and our own observations of the genus. Figure 2 and Supporting Information, Figs S1–S5 show detailed illustrations of the muscles *in situ* for at least one representative of each major clade in the darter phylogeny.

Adductor mandibulae

The adductor mandibulae segmentum fascialis is the primary jaw-closing muscle and is divided into an upper malaris and lower rictostegalis in most darter species examined, as in *Perca* (Fig. 2). The tendonous connections of the muscle are not well resolved through diceCT, but the pars malaris originates on the preopercle and hyomandibula, while the rictostegalis originates on the preopercle and posterior portions of the quadrate, and appears to insert on the coronoid process in most species. The adductor mandibulae complex showed the greatest overall anatomical variation, as well as the most significant departures from previous non-darter descriptions (Figs 3, 4). *Perca fluviatilis* (Osse, 1969) and *Perca flavescens* (Mitchill, 1814)

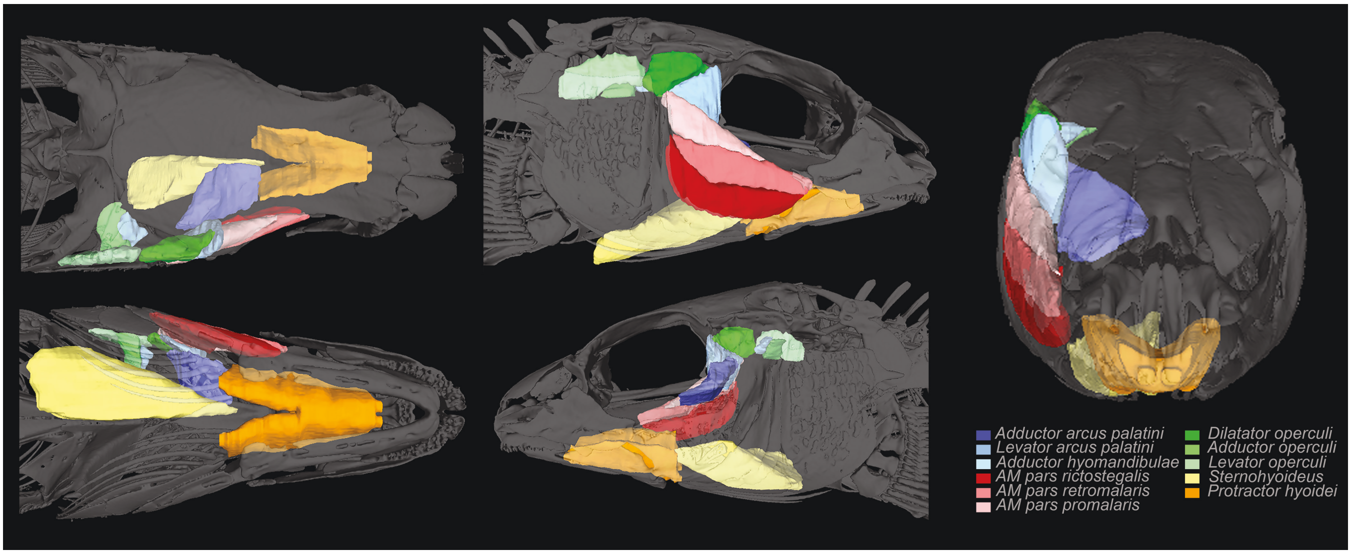

Figure 2. Reconstruction of cranial musculature (major adductors and abductors) in logperch, *Percina caprodes* (MTSUZ-12, 90.3mm SL) from a diceCT scan. Skull from an unstained CT scan shown as a transparent overlay. Views clockwise from top left: dorsal, right (lateral), anterior, left (medial) and ventral. See Supporting Information, Figs S1–S5 for additional species).

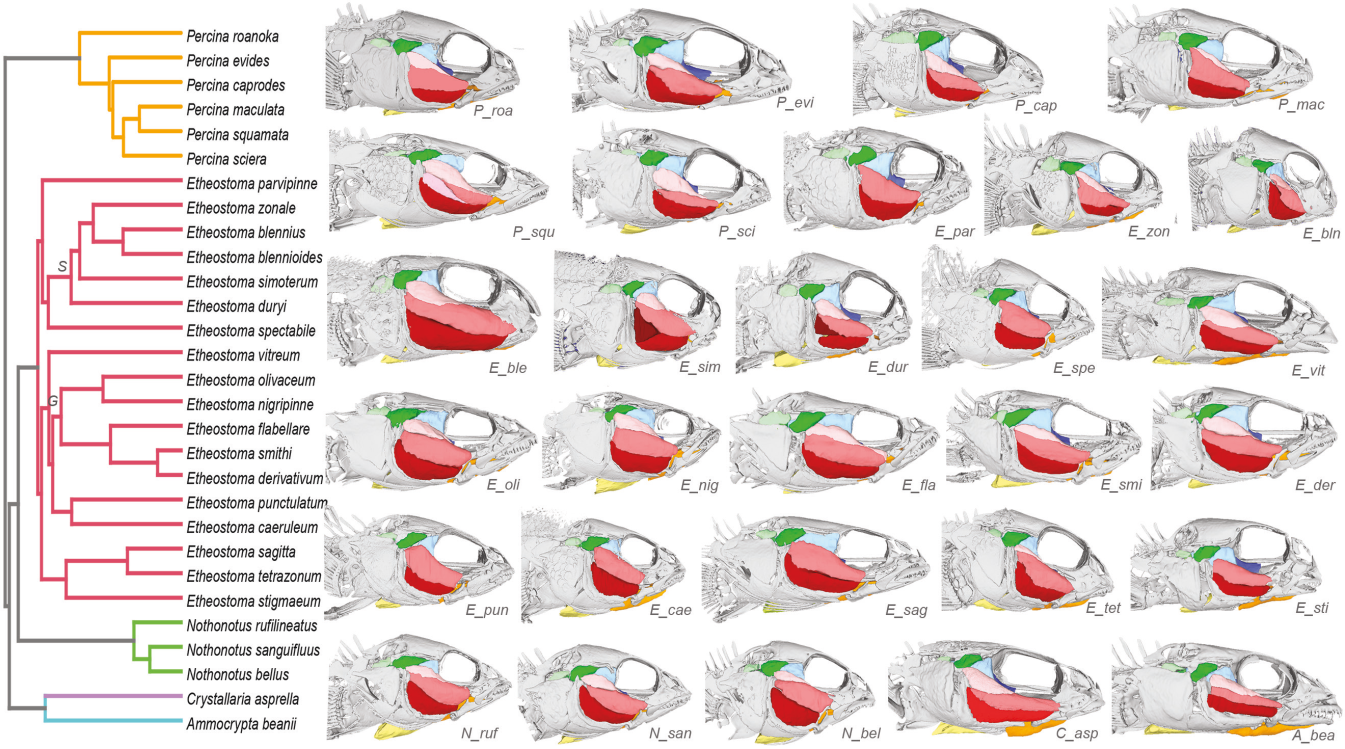

Figure 3. Variation in major feeding musculature of the darter cranium from diceCT scans of 30 species. Muscle colour-coding matches legend in Figure 2. Phylogeny from Arbour and Stanchak (2021), with branches coloured by genus, 'S' and 'G' indicate the *Simoperca* and *Goneaperca* subgenera of *Etheostoma*, respectively.

possess mostly parallel fibres in the AM segmentum fascialis, with fibres directed from the lower jaw/maxilla to the margin of the preoperculum (Fig. 4A), and an overall fusiform or convergent muscle shape. By comparison, many darter species show variation in the orientation and pennation of the muscle fibres of both the malaris and rictostegalis (Figs 4B–F, 5). All darters showed more vertically oriented fibres in the AM pars rictostegalis, with fibres frequently directed towards the 'midline' between the rictostegalis and malaris segments (Fig. 4B–F). The muscle appears to be unipennate, though connective tissue and tendons were not easily visualized using diceCT. This shift in fibre orientation probably produces shorter muscle fibres compared with the orientation seen in yellow perch, though this was not quantified. This portion of the AM was typically the largest, ranging from 30% to 50% of the total AM muscle volume.

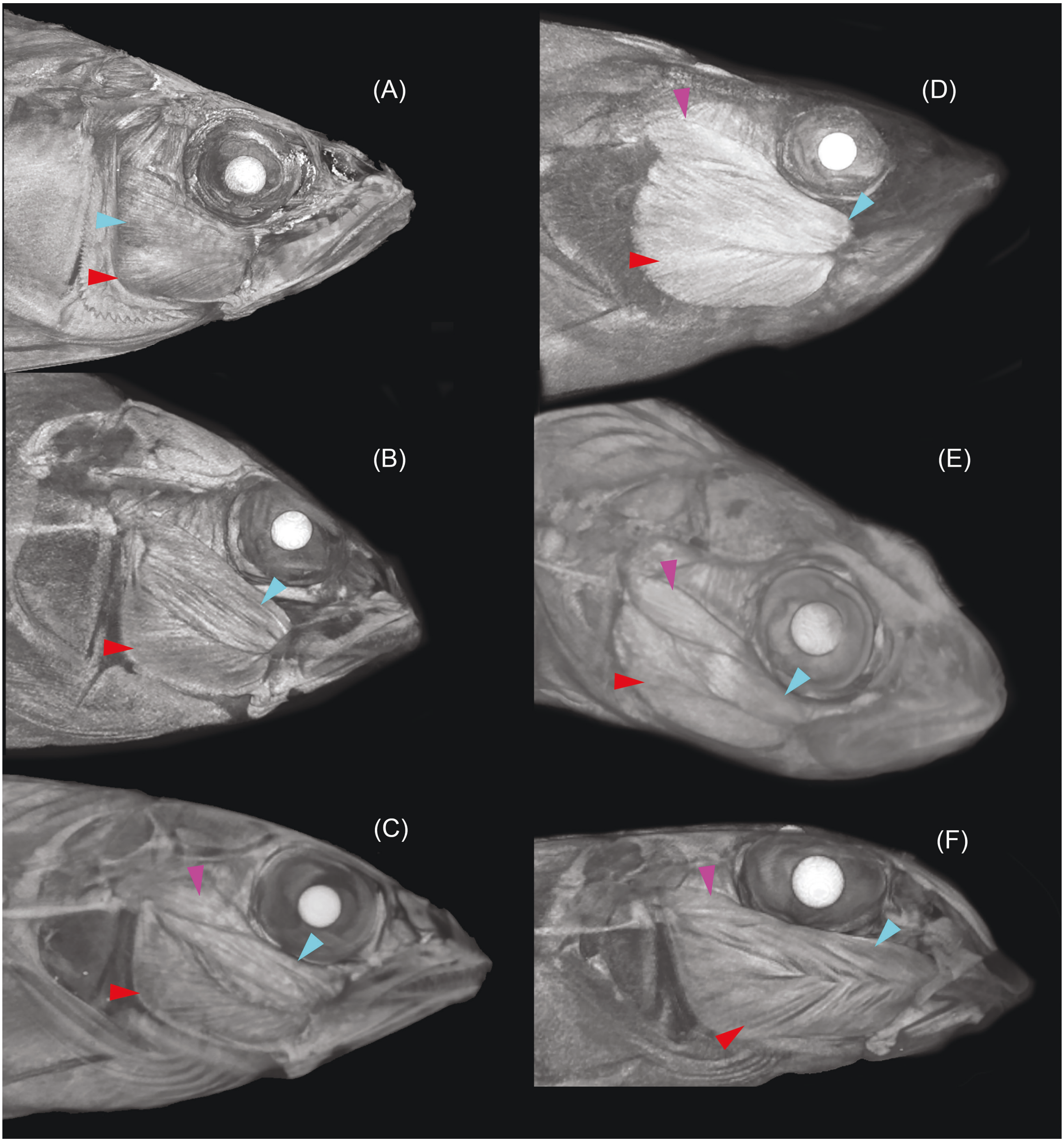

Figure 4. Variation in adductor mandibulae divisions and fibre architecture in Percidae. A, yellow perch, *Perca flavescens*; B, orangethroat darter, *Etheostoma spectabile*; C, bloodfin darter, *Etheostoma sanguifluus*; D, blackfin darter, *Etheostoma nigripinne*; E, stone darter, *Etheostoma derivativum*; F, greenside darter, *Etheostoma blennioides*. Blue arrows = adductor mandibulae pars malaris or retromalaris; magenta arrow = adductor mandibulae pars promalaris; red arrow = adductor mandibulae pars rictostegalis or rictalis (and see text for description of relative positions of these divisions). B–F, illustrate the change in rictostegalis fibre orientation compared to *Perca*. C–F, show the additional division of the upper pars promalaris portion, especially in (D), while (E) illustrates the medial positioning of the anterior pars promalaris. F, shows the additional reorientation of the fibres of the retromalaris towards the intersection with the rictostegalis.

In most darters examined (23 species; Fig. 3), the AM malaris fibres originating on the hyomandibula were distinct from those inserting on the preopercle, with some showing distinct differences in fibre orientation (Fig. 4; blue vs. magenta arrows). We designated the portion inserting on the hyomandibula the 'AM pars promalaris' and those inserting on the preopercle the 'AM pars retromalaris' (Figs 2–4). Typically, the promalaris portion becomes increasingly medial to the retromalaris anteriorly (Fig.

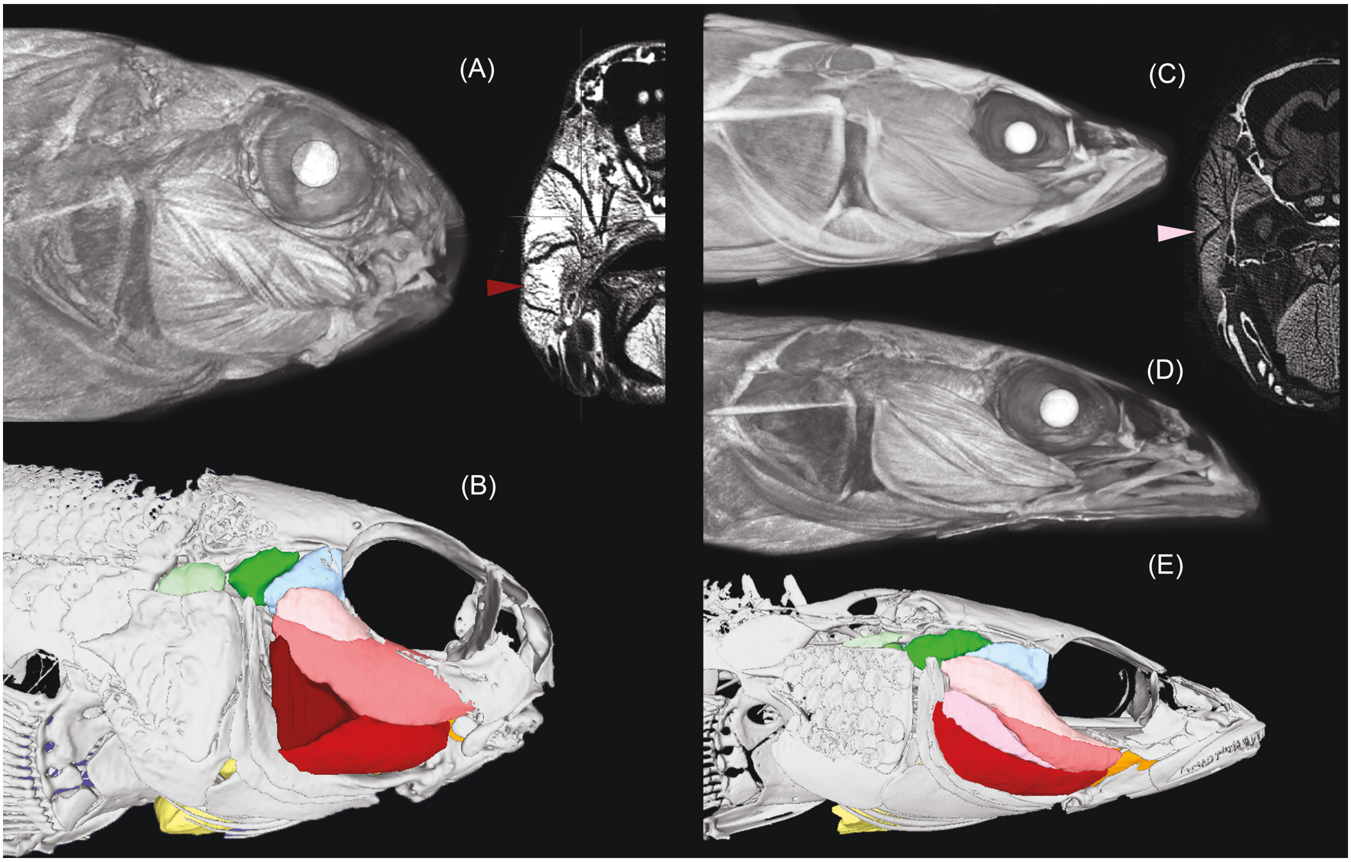

Figure 5. Darter species with highly modified adductor mandibulae. A, B, snubnose darter, *Etheostoma simoterum*, with separate rictalis and stegalis portions. Coronal slice from diceCT image stack shows the large and 'uncovered' AM pars stegalis (dark red arrow). C–E, AM of the subgenus *Swainia*, in *Percina squamata* (C) and *Percina nasuta* (Mulberry River) (D). Light red arrow shows the position of the novel pars 'ectomalaris'. E, reconstruction of *Percina squamata*. B, reconstruction of *Etheostoma simoterum*. Colours of 3D muscle models follow follows that of Figure 2 and 3.

4E), and sits entirely dorsal to the retromalaris posteriorly. This portion is most (superficially) obvious in members of the subgenus *Goneaperca* (e.g. Fig. 4D, E. *nigripinne* Braasch and Mayden, 1985), where the 'promalaris' can be extended into a distinct lobe. Overall, this division ranged from 10% to 35% of total AM volume. In some species like *E. blennioides* and *E. simoterum* (Cope, 1868) the promalaris and retromalaris show drastically different fibre orientations compared to the promalaris (Fig. 5; Supporting Information, Fig. S6). The retromalaris portion also shows more vertically oriented muscle fibres in species in the subgenus *Simoperca*, particularly *E. blennioides* and *E. simoterum* (Supporting Information, Fig. S6). In *Nothonotus* and in *E. simoterum* the AM pars retromalaris appears to possess two distinct fibre bundles (Fig. 4), possibly with portions inserting on different parts of the connective tissue connecting the lower jaw and maxilla. The AM pars retromalaris in *E. simoterum* also appears to be bipennate (Fig. 5A).

In *Percina squamata* (subgenus *Swainia)*, we found a novel portion between the malaris and rictostegalis portions (Fig. 5C–E), which we denoted as the 'ectomalaris'. This long and narrow bundle of fibres runs parallel to the body axis and remains superficial to the rest of the AM complex for the duration of its length. An additional scan (which was not segmented or included in muscle volume analyses) of a specimen of *Percina nasuta* (Bailey, 1941; YPM ICH 34344, which is also another member of the subgenus *Swainia*), also shows this novel segment, as well as a more noticeable division between the promalaris and retromalaris portions than in the scan of *P. squamata*. We found no similar division in any other members of *Percina* (or other darters), and this may represent a novel feature of the subgenus (Fig. 2).

While most darters show a combined rictostegalis (as in *Perca*) this muscle is divided into a larger, lateral AM rictalis and smaller, largely medial AM stegalis portion in seven species examined, predominantly in *Nothonotus* and *Etheostoma*–*Simoperca* (Table S2). The small stegalis in *Nothonotus* and *E. blennius* originate on the metapterygoid or the most ventral portions of the hyomandibula. While the AM stegalis is small in most species (<5% total AM volume), among the blunt-faced species in *Simoperca*, the stegalis reaches it largest proportional sizes, representing 10%–25% of the total volume of the AM complex. Furthermore, in *E. simoterum* and *E. duryi* (Henshall, 1889), the stegalis widens and posteriorly is no longer medial to the AM pars malaris or rictalis, extending to the 'cheek' between these two portions (Fig. 5) and inserting on the preopercle. Among these 'snubnose' darters the AM complex appears to become the most complex in fibre orientations among the darters examined (Fig. 5).

Suspensorium

The levator arcus palatini originates on the sphenotic behind the orbit and inserts on the hyomandibula or extends on to the metapterygoid (Fig. 2; Supporting Information, Figs S1–S5), with fibres largely parallel (Fig. 4). The lower portion sits medial to the AM pars malaris (Figs 2, 3). In species of *Simoperca* with large levator arcus palatini (e.g. *Etheostoma blennius* and *Etheostoma zonale* Cope, 1868), the upper body of the hyomandibula is steeply angled, with a sharp bend into the more vertical lower body, creating an indented region (Supporting Information, Fig. S7). The adductor arcus palatini originates on the posterior parasphenoid, extending dorsally on to the ascending process of the parasphenoid and the prootic (Fig. 2; Supporting Information, Figs S1–S5), and inserts on the medial surface of the anterior arm of the hyomandibula and the dorsal margin of the metapterygoid. The adductor hyomandibula is a small bundle of laterally oriented fibres, which is more visibly differentiated than in *Perca* from the adductor operculi (see below). It originates on the prootic and ventral pterotic and inserts on the medial surface of the posterior arm of the hyomandibula just ahead of the joint with the opercle.

Operculum

The dilatator operculi is the major abductor and rotator of the opercular series. It is pennate and originates broadly on the pterotic, dorsal to the levator arcus palatini, and inserts medially on the anteriodorsal process of the opercle just above its joint with the hyomandibula (Fig. 2; Supporting Information, Figs S1–S5, S7). The levator operculi is a single unit across all darters examined, in contrast with the two divisions observed in *Perca fluviatilis* (Osse, 1969). It is a laterally thin and posterioventrally broad muscle with parallel fibres, originating caudally on the pterotic and inserting on, and dorsally to, the medial ridge of the opercle. In more elongate skulls in *Goneaperca*, *Nothonotus*, and *Percina*, the levator opercli is more angled anterio-posteriorly, while in more rounded heads it is more vertical (Fig. 2; Supporting Information, Figs S1–S5). The adductor operculi is a long, narrow and medially oriented muscle originating on the exocciptal and ventral pterotic and attaching to the operculum below the anterior end of the medial ridge, typically ventral to the levator operculi. It sits immediately posterior to the adductor hyomandibulae.

Hyoid apparatus

In prior anatomical descriptions of *Perca*, the muscle connecting the jaw and hyoid is referred to as the geniohyoideus, but we follow the recent literature in referring to this as the protractor hyoidei. As in *Perca*, this muscle connects two movable elements, and so we refer to these as attachments rather than origin and insertion (Osse 1969). The anterior paired protractor hyoidei merge and attach near the medial surface of the symphysis of the lower jaw (Fig. 2; Supporting Information, Figs S1–S5). Fibres run both above and below the small horizontal intermandibularis in most species examined. The posterior protractor hyoidei attaches to the mid-lateral surface of the ceratohyal, with fibres extending ventral to the second branchiostegal ray, with some extending to the third. The sternohyoideus is a large, wedge-shaped muscle with three myosepta. The third myoseptum originates on, and lateral to, the broad and angled symphysis of the cleithrum, and is partially contiguous with the hypaxials laterally, and inserts laterally on the urohyal (Fig. 2; Supporting Information, Figs S1–S5).

Comparative analysis of darter muscle size

The first axis of the phylogenetically corrected principal component analysis (pPC1, 56.7% of variation) represents a gradient between species with comparatively large cranial muscle volumes (after size correction) possessing positive pPC1 scores and those with small muscle volumes with negative pPC1 scores (Fig. 6). We observed a strong phylogenetic bias in the distribution of pPC1 scores—low overall muscle size was associated with basal lineages in *Ammocryta*, *Crystallaria*, and *Percina*, while *Etheostoma* and *Nothonotus* possessed pPC1 scores indicative of large (size-corrected) muscle volumes. However, a small number of species of *Etheostoma* appear to show evolutionary trajectories towards low PC scores/lower muscle volumes (e.g. *E. vitreum* Cope, 1970, *E. parvipinne* Gilbert and Swain, 1887, and *E. stigameum* Jordan, 1877).

Comparatively, pPC2 (14.5% of total variation) describes a trade-off between species with larger AM and adductor hyomandibulae volumes (negative pPC2) compared with those with larger volumes of the arcus palatini complex (suspensorium) and the opercular muscles (dilatator, levator, and adductor opercula). Those muscles loading strongly to positive pPC2 scores were (with exceptions) largely levators/abductors responsible for expanding the buccal and opercular cavities (suction feeding), while those associated with negative pPC2 scores were largely biting muscles (AM malaris and AM rictostegalis). Species with high pPC1 scores (larger muscle volumes) overall showed higher variation in pPC2 scores, primarily within species of *Etheostoma*. In particular, species of the *Simoperca* subgenus of *Etheostoma* possessed both the highest and lowest values of pPC2 (Fig. 6, blue points).

Macroevolutionary trends

DTT analysis of PC1 showed a significantly negative MDI value indicative of an early burst of evolution (MDI = –0.176, *P* = .022; Fig. 7). This was consistent with the strong split observed between the *Etheostoma–Nothonotus* clade and species of *Ammocrypta*, *Crystallaria*, and *Percina* species in PC1 scores. Comparatively, PC2 showed a positive MDI value (MDI = 0.376, *P* = .983) with subclade disparity peaking around the diversification of the *Simoperca* subgenus and afterwards returning to BM expectations (Fig. 7). We also detected a major, strongly supported shift in adaptive peaks at the base of the *Etheostoma–Nothonotus* clade (based on an analysis of all muscle residual volumes; Fig. 8). Across all muscles, this shift in adaptive optimum was associated with higher overall muscle volume residuals (higher proportional muscle sizes). We also detected shifts on the branches corresponding to *E. vitreum* and *E. stigmaeum* (moderate bootstrap support), both of which were found to be convergent on the ancestral adaptive optimum shared between *Ammocrypta*, *Crystallaria*, and *Percina*.

DISCUSSION

Signals of ecological adaptation

Our diceCT approach revealed several significant patterns in the macroevolutionary trends of darter fish cranial muscle diversification. Most notably, muscle investment in darters appears to have undergone an early divergence between the clade of *Etheostoma–Nothonotus* (greater volumes) and the paraphyletic

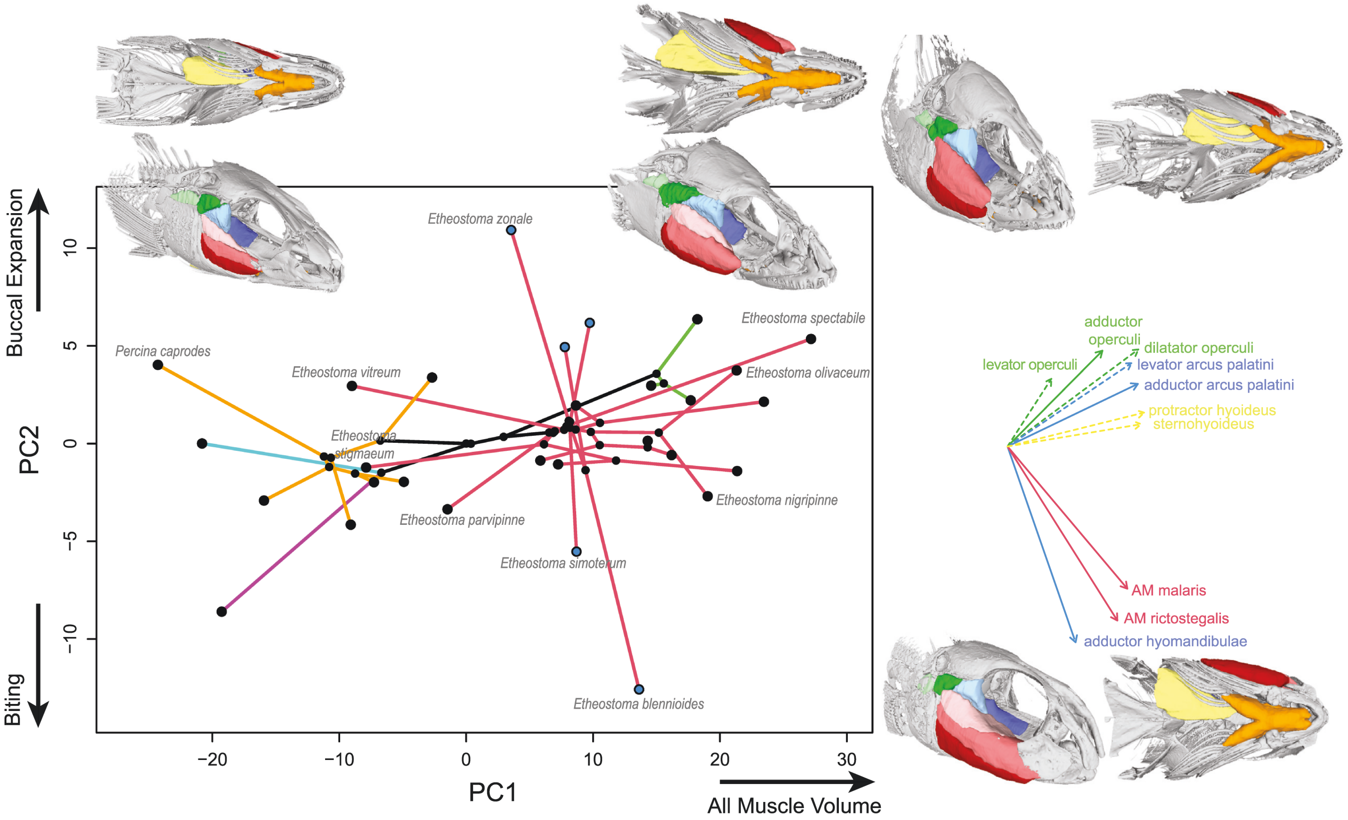

Figure 6. Phylogenetic principal component analysis of size-corrected muscle volumes from 29 darter species. Left, phylomorphospace of darter muscle size PCA, point show PC scores and lines indicate evolutionary branches. Branches coloured by genus, see Figure 3. Skull and muscle reconstructions illustrate the major trends on each axis. PC1, *Etheostoma olivaceum* (right) and *Percina caprodes* (left). PC2, *Etheostoma spectabile* (top) and *Etheostoma blennioides* (bottom). Right, eigenvectors illustrating the major relationships between the residual muscle volumes. Arrow colour: red = biting muscles (AMs), blue = suspensorium (AAP, LAP, AH), yellow = hyoid (PH and S), green = opercular series (LO, AO, DO). Solid arrows indicate adductors and dashed indicate abductors/levators.

Percina–Ammocrypta–Crystallaria group (lesser volumes). Species of *Percina* have been previously referred to as the 'midwater darters', as unlike many other darter species, some still possess rudimentary swim bladders and thus are able to swim up above the substrate (Collette 1963, Page 1983, Carlson and Lauder 2011, Bossu and Near 2015). Specialized foraging in *Percina* (e.g. rock flipping) often makes use of this greater mobility (Carlson and Wainwright 2010). These species also tend to be found in large rivers, often with 'softer' substrates like sand, silt, and detritus (Schroth 2024; Page 1983, Etnier and Starnes 1993, Ciccotto and Mendelson 2016), although some are found in riffles as well (but are largely hyperbenthic in that case). Similarly, *Ammocrypta* and *Crystallaria* (the sand darters) live on soft and sandy substrates in large rivers, though rest directly on the bottom. In contrast, species of *Etheostoma* and *Nothonotus* are strongly benthic, lacking a swim bladder and sitting directly on the substrate. These species are more likely to inhabit the fast-flowing waters of small- to medium-sized creeks and streams, often over hard substrates like limestone, shale, and varying degrees of cobblestone, gravel, and bedrock. Species of *Etheostoma* and *Nothonotus* may be more likely to encounter prey with strong attachments in these more vigorous and rocky river systems. We suggest that the strong adaptive signal in overall muscle size that divides these two clades may result from adaptations to foraging in different current and substrate environments. Our l1ou results also support this assessment. The species identified as having converged on the 'basal' adaptive peak occupied by *Ammocrypta*, *Crystallaria*, and *Percina*, included *Etheostoma vitreum* and *Etheostoma stigmaeum*, which are among the lesser number of species of *Etheostoma* that occupy sandy pools. Interestingly, Carlson and Wainwright (2010) also placed *E. vitreum* as convergent on *Ammocrypta* and *Crystallaria* in feeding ecomorphology. Adaptation to benthic living in rocky and fast-water environments also appears to have facilitated diversification along a traditional suction-biting feeding spectrum in the *Etheostoma–Nothonotus* clade. While Carlson and Wainwright (2010) have suggested that the small, downturned jaws of species of *Simoperca* (*Etheostoma*) (designated as the 'manipulator' foraging group) may be well adapted for biting prey on the surface of rocks, *Simoperca* showed high diversification across PC2, which we suggest is associated with biting–suction trade-offs. Indeed, the small gape and, in some cases, more mobile premaxilla, may aid in suction feeding in some species of this subgenus.

Functional and developmental correlates of darter muscle diversification

Darters show multiple changes in cranial muscle architecture, in particular in the adductor mandibulae complex, in comparison

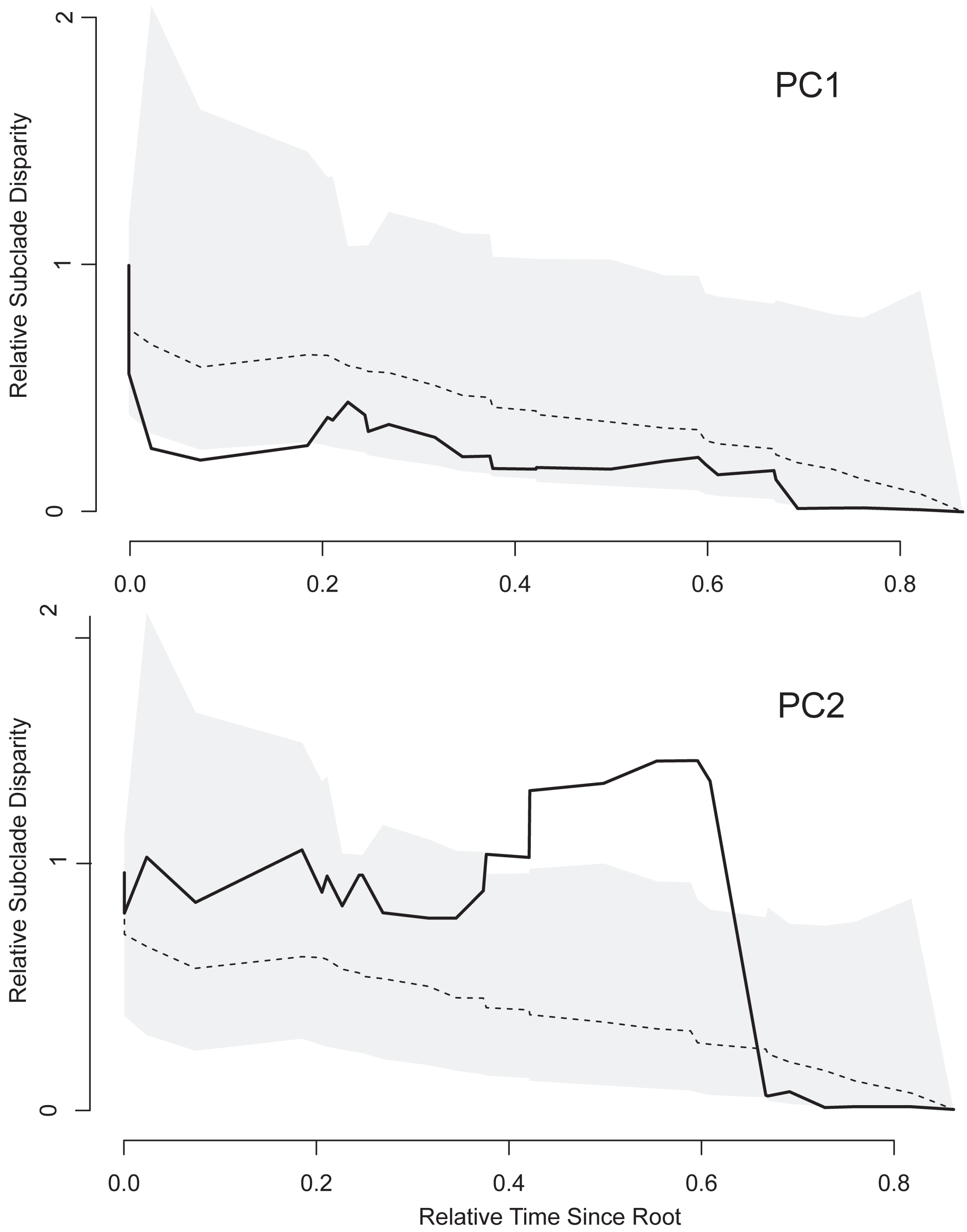

Figure 7. Results of DTT analysis of PC1 and PC2 of darter size-corrected muscle volumes. Solid line shows observed subclade disparity, the shaded polygon shows the 95% confidence interval of BM simulated subclade disparities and the dotted line shows the mean of all BM simulations.

with the previously documented anatomy of *Perca* (Osse, 1969) and *Sander* (Elshoud-Oldenhave, 1979). Many of the observed changes in fibre orientation in the AM pars rictostegalis and pars malaris would produce shorter overall muscle fibre lengths compared to the anatomy of *Perca* (Osse, 1969), and possibly proportionately higher maximum force production in the muscle (as muscle force scales with muscle size/fibre length, among other terms). In particular, the two pennate divisions of the AM (pars malaris and rictostegalis) forming a 'chevron-shaped' fibre orientation of *E. blennioides* (the species with the largest proportional AM muscles; Figs 3, 4, 6 and S6) show striking resemblance to groups like marine butterflyfishes (Copus and Gibb

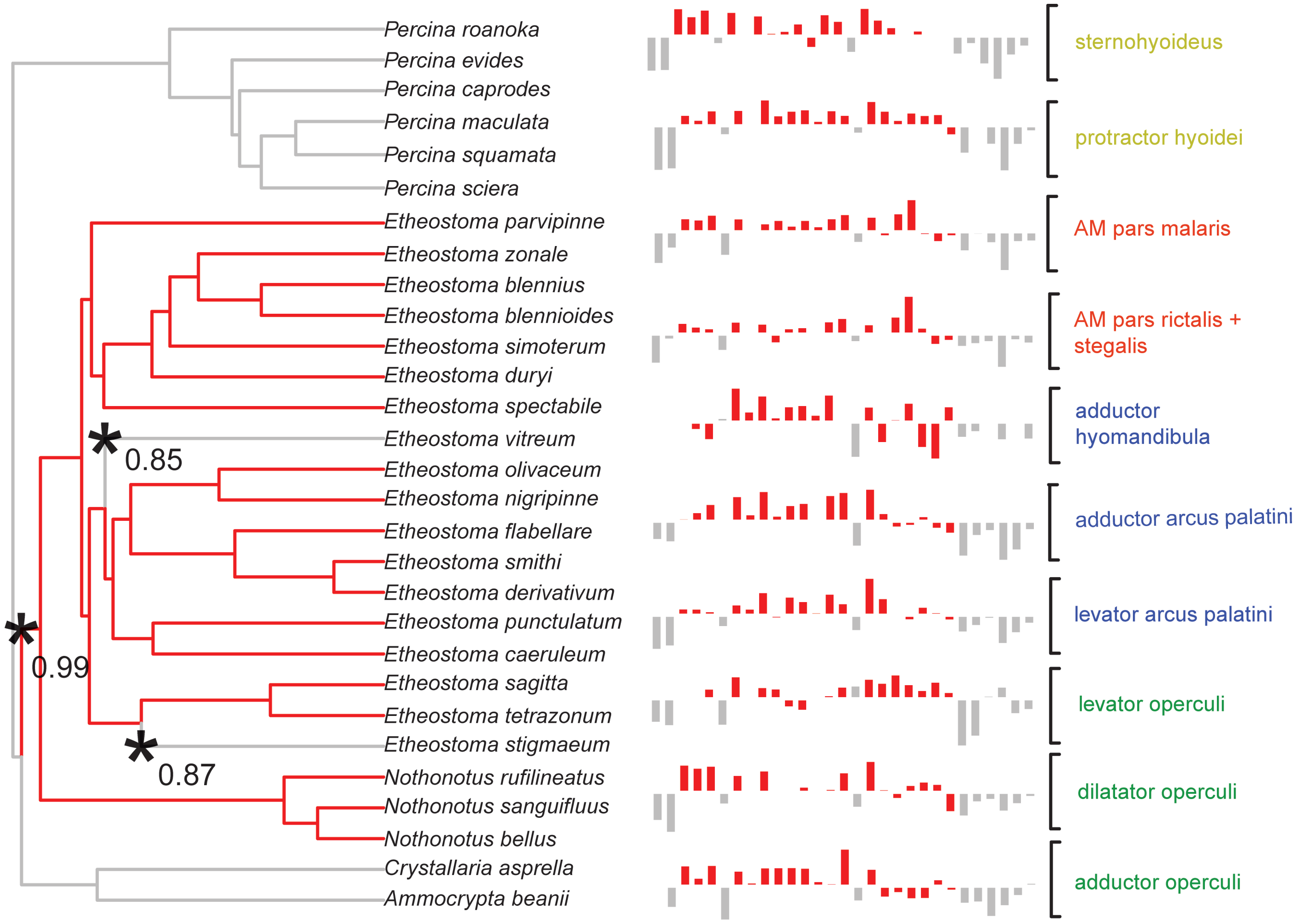

Figure 8. Results of l1ou analysis of darter size-corrected muscle volumes. * indicates the location of a detected shift in adaptive optima, and values indicate the bootstrap support for each shift. Bars illustrate the relative muscle volume residuals for each muscle (rotated 90° clockwise from phylogeny labels). Muscle names are coloured by cranial system: red = lower jaw adductors (AMs), yellow = hyoid apparatus (S and PO), blue = suspensorium/hyomandibula (AH, AAP, LAP), green = opercular series (DO, LO, AO).

2013), which have an AM complex adapted to forceful biting of the upper and lower jaws.

Division of the AM malaris into upper/medial and lower/lateral segments was found in the majority of species and across all darter genera examined. The functional consequences of this division are unclear, but the modularization of the AM malaris, which connects to both the maxilla and lower jaw, may help to fine-tune movements of the oral jaws, or allow mechanical specialization (e.g. short fibres and high force potential) in the two divisions. Though typically the pars retromalaris was often larger, the pro-malaris showed the largest proportional contribution to total AM mass in species with either moderately upturned mouths (e.g. *E. nigripinne*) or elongate heads (e.g. *P. squamata* and *P. sciera* Swain, 1883). Fibre orientation differed dramatically between the promalaris and retromalaris in some species with more rounded faces (Fig. 3; Supporting Information, Fig. S6), but typically were similar in species with elongate heads or large, upturned mouths (e.g. *E. nigripinne*). The functional role of the pars promalaris, and the extent of kinematic specialization between the divisions of the AM malaris, may, therefore, vary across the darter clades examined.

The long and narrow novel portion of the AM muscle in *Swainia* (AM pars ectomalaris), shows long, parallel fibres that are less ideal for strong force production. The small overall size of this segment also indicates a low contribution to bite force potential. Long fibre lengths are associated with wide gape angles and long jaws in other vertebrate groups (Santana 2016), and produce faster shortening speeds. This segment of the AM may contribute to fast movements or movements at wide gapes, while inserting the snout into rocky crevices. The 'probers' of the subgenus *Swainia* have long and narrowed heads and have significant changes in the kinematics of the oral jaws, with the upper jaw remaining fixed in a protruded state, unaffected by movement of the lower jaw, and are supported by elongated bones of the snout (Carlson and Wainwright 2010). These additional divisions of the AM malaris may be related to the reconfiguration of these typical teleost oral jaw kinematics. Live-feeding kinematic studies would be highly beneficial in investigating these innovative patterns in jaw musculature.

Divergence along a possible biting–suction feeding gradient (PC2) was most prominent in the 'snubnosed' subgenus *Simoperca*. Interestingly, this clade has been previously documented for possessing divergent head allometries; adult head shapes were more variable than juvenile head shapes, and the direction of change varied across species (Goodwin and Arbour 2022). Such developmental lability in head shape may be associated with the reapportioning of space in the skull to accommodate changing proportions of jaw vs. opercular/suspensorium muscle groups.

Innovation in darter biting muscle anatomy

The variety of AM divisions in the AM complex in darters was an unexpected observation (in the authors' experience). Such diversity in AM structure was greater than previously observed in many trophically diverse adaptive radiations, like wrasses and parrotfish (Labridae) and African or Neotropical cichlids (Cichlidae) (Westneat 2003, Arbour and López-Fernández 2018, Gobalet 2018). Though not as extreme in its variation, the complexity of shifts in darter AM structure is more akin to that observed among some clades of Tetraodontiformes, which show repeated 'duplications' of the AM pars malaris and AM pars rictalis (Friel and Wainwright 1999, Konstantinidis and Harris 2011), though chiefly among the AM pars malaris in darters. This diversity contrasts with the low diversity in ecomorphological traits observed by Carlson and Wainwright (2010); however, this prior study focused only on linear measures of the oral jaws (and one hyoid measurement) and no muscle characteristics.

The diminutive size and elongate body shape of darter fishes is probably an adaptation to their rheophilic lifestyle, allowing them to maintain position within the benthic boundary layer of slower moving waters (Carlson and Lauder 2010, 2011, Lujan and Conway 2015). As such, overall body form may be under strong selective constraints. Adaptations typically associated with suction feeding and biting may include deepening of the body to accommodate larger body musculature (epaxials for suction feeding), sternohyoideus (hyoid depression) or cheek muscles (Westneat 1994, Wainwright *et al.* 2004, Arbour and López-Fernández 2014, Camp *et al.* 2015, Hulsey, Holzman and Meyer 2018). If increased body height would be detrimental to drag resistance in high current waters, this may make alternate adaptations to increase bite force (e.g. decreasing fibre length, changing divisions/orientations of the muscles) more advantageous by comparison. The body musculature of fish contributes significantly to suction feeding in many species of fish (Carroll *et al.* 2004, Camp and Brainerd 2014). The narrow body in darters and the small supraoccipital crests (attachment of epaxials that rotate the neurocranium upwards) limit the mechanisms by which body-driven suction may be optimized, by limiting the size of the epaxials connecting to the cranium (Carroll *et al.* 2004, Camp and Brainerd 2014, Camp *et al.* 2020).

Biting feeding strategies are associated with increased head-shape diversification across fishes, although suction feeders see increased rates of evolution in kinematic traits in some studies (Collar *et al.* 2014, Corn *et al.* 2021, Corn and Friedman 2022). Carlson and Wainwright (2010) noted that changes in the skull shape and kinematics of many darters limit the potential to optimize suction feeding, such as decreases in the mobility of the premaxilla in 'rock flippers' and 'probers'. In particular, we found that innovations in muscle architecture were associated with morphological extremes among darter species. The most 'derived' forms observed were among the most flat-faced species (*E. simoterum*) and the most elongated (*P. squamata*). Body-shape constraints that disadvantage suction feeding and advantage biting foraging strategies in darters may have contributed to the high variability in head shape and, concomitantly, cranial muscle architecture. It has also been hypothesized that miniaturization in fishes and other animals is associated with morphological innovation/novelties (Weitzman and Vari 1988, Hanken and Wake 1993), and it is possible that the small size of darters is related to the lability in AM structure. We note that both 'large' (*E. blennioides*) and 'small' (*E. simoterum*) darters showed strong divergence in AM structure compared with *Perca* and *Sander*, although both these fishes represent small species compared with the non-darter percids (*Percinae* and *Luciopercinae*, *Percidae*).

CONCLUSION

Contrast enhanced CT scanning approaches have provided a number of anatomical insights in vertebrate clades, including bats, birds, rodents, and snakes (Santana 2018, Dickinson *et al.* 2019, Santana *et al.* 2019, Sullivan *et al.* 2019, Cox *et al.* 2020, Widrig *et al.* 2023, de Oliveira *et al.* 2024), among others, and in taxonomically limited (i.e. small numbers of species) applications in fishes (e.g. Brocklehurst *et al.* 2019, Camilieri-Asch *et al.* 2020). DiceCT approaches may be particularly fruitful in the study of fish anatomy given the complexity of kinematics and musculature, especially in teleost fishes with mobile skulls. Here we demonstrate its utility in a macroevolutionary context (i) across a comparatively 'high' number of species and (ii) in small and rare fishes where traditional dissection would be impractical or inadvisable. In darters, digital dissection of feeding-associated muscles shows clear phylogenetic divergence across the sand-darter and midwater-darter clade vs. benthic darters, and patterns suggestive of ecological adaptation. Further studies expanding taxonomic sampling and directly incorporating data on substrate type would be beneficial in assessing these putative adaptive signals. We note that while some darters species are rare, many others, especially cosmopolitan species like greenside darters (*Etheostoma blennioides*), are frequently found in North American ichthyological collections. Despite this, many novel anatomical features described here have not (to the knowledge of the authors) been previously documented. We emphasize how diceCT studies may provide unique insights into even 'common' species in natural history institutions, and we believe this supports the utility of projects like the NSF-funded 'oVert' initiative in documenting and digitizing museum specimen collections.

SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

ACKNOWLEDGEMENTS

We are grateful to Hernan Lopez Fernandez and Randy Singer (University of Michigan, LSA Museum of Zoology), Tom Near and Greg Watkins-Colwell (Yale Peabody Museum) for access to specimens under their care. We appreciate the assistance, access to equipment, and training from Sasidhar Uppuganti, Jarrod True, and Jeff Nyman at the Vanderbilt Center for Small Animal Imaging (Vanderbilt Institute of Imaging Sciences). We are grateful for the comments provided by two reviewers.

FUNDING

This work was supported by an NSF BIO-DEB grant (#2135927) and a Middle Tennessee State University MTSU FRCAC grant (Office of Research and Sponsored Programs) to JHA.

DATA AVAILABILITY

Muscle volumes and body sizes are provided in the Supporting Information. Phylogeny is available through Arbour and Stanchak (2021). Models of skulls and muscles are available for viewing on Sketchfab (https://sketchfab.com/MTSUichthyology). Original diceCT scan data, and resulting 3D models are available through morphosource.org (project ID: 000656696).

REFERENCES

Arbour JH, López-Fernández H. Adaptive landscape and functional diversity of Neotropical cichlids: implications for the ecology and evolution of Cichlinae (Cichlidae; Cichliformes). *Journal of Evolutionary Biology* 2014;**27**:2431–42. https://doi.org/10.1111/jeb.12486

Arbour JH, López-Fernández H. Continental cichlid radiations: functional diversity reveals the role of changing ecological opportunity in the Neotropics. *Proceedings Biological Sciences* 2016;**283**:20160556. https://doi.org/10.1098/rspb.2016.0556

Arbour JH, López-Fernández H. Intrinsic constraints on the diversification of Neotropical cichlid adductor mandibulae size. *Anatomical Record* 2018;**301**:216–26.

Arbour JH, Stanchak KE. The little fishes that could: smaller fishes demonstrate slow body size evolution but faster speciation in the family Percidae. *Biological Journal of the Linnean Society* 2021;**134**:851–66. https://doi.org/10.1093/biolinnean/blab125

Baverstock H, Jeffery NS, Cobb SN. The morphology of the mouse masticatory musculature. *Journal of Anatomy* 2013;**223**:46–60. https://doi.org/10.1111/joa.12059

Bossu CM, Near TJ. Ecological constraint and the evolution of sexual dichromatism in darters. *Evolution* 2015;**69**:1219–31. https://doi.org/10.1111/evo.12655

Brocklehurst R, Porro L, Herrel A *et al.* A digital dissection of two teleost fishes: comparative functional anatomy of the cranial musculoskeletal system in pike (*Esox lucius*) and eel (*Anguilla anguilla*). *Journal of Anatomy* 2019;**235**:189–204. https://doi.org/10.1111/joa.13007

Camilieri-Asch V, Shaw JA, Yopak KE *et al.* Volumetric analysis and morphological assessment of the ascending olfactory pathway in an elasmobranch and a teleost using diceCT. *Brain Structure and Function* 2020;**225**:2347–75. https://doi.org/10.1007/s00429-020-02127-1

Camp AL, Brainerd EL. Role of axial muscles in powering mouth expansion during suction feeding in largemouth bass (*Micropterus salmoides*). *The Journal of Experimental Biology* 2014;**217**:1333–45. https://doi.org/10.1242/jeb.095810

Camp AL, Brainerd EL. Reevaluating musculoskeletal linkages in suction-feeding fishes with x-ray reconstruction of moving morphology (XROMM). *Integrative and Comparative Biology* 2015;**55**:36–47. https://doi.org/10.1093/icb/icv034

Camp AL, Roberts TJ, Brainerd EL. Swimming muscles power suction feeding in largemouth bass. *Proceedings of the National Academy of Sciences of the United States of America* 2015;**112**:8690–5. https://doi.org/10.1073/pnas.1508055112

Camp AL, Olsen AM, Hernandez LP *et al.* Fishes can use axial muscles as anchors or motors for powerful suction feeding. *Journal of Experimental Biology* 2020;**223**:jeb225649. https://doi.org/10.1242/jeb.225649

Carlson RL, Lauder GV. Living on the bottom: kinematics of benthic station-holding in darter fishes (Percidae: Etheostomatinae). *Journal of Morphology* 2010;**271**:25–35. https://doi.org/10.1002/jmor.10776

Carlson RL, Lauder GV. Escaping the flow: boundary layer use by the darter *Etheostoma tetrazonum* (Percidae) during benthic station holding. *Journal of Experimental Biology* 2011;**214**:1181–93. https://doi.org/10.1242/jeb.051938

Carlson RL, Wainwright PC. The ecological morphology of darter fishes (Percidae: Etheostomatinae). *Biological Journal of the Linnean Society* 2010;**100**:30–45. https://doi.org/10.1111/j.1095-8312.2010.01417.x

Carroll AM, Wainwright PC. Energetic limitations on suction feeding performance in centrarchid fishes. *The Journal of Experimental Biology* 2009;**212**:3241–51. https://doi.org/10.1242/jeb.033092

Carroll AM, Wainwright PC, Huskey S *et al.* Morphology predicts suction feeding performance in centrarchid fishes. *The Journal of Experimental Biology* 2004;**207**:3873–81.

Ciccotto PJ, Mendelson TC. The ecological drivers of nuptial color evolution in darters (Percidae: Etheostomatinae). *Evolution* 2016;**70**:745–56. https://doi.org/10.1111/evo.12901

Collar DC, Wainwright PC, Alfaro ME *et al.* Biting disrupts integration to spur skull evolution in eels. *Nature Communications* 2014;**5**:1–9.

Collette BB. The subfamilies, tribes, and genera of the Percidae (Teleostei). *Copeia* 1963;**1963**:615–23. https://doi.org/10.2307/1440961

Copus JM, Gibb AC. A forceful upper jaw facilitates picking-based prey capture: biomechanics of feeding in a butterflyfish, Chaetodon trichrous. *Zoology* 2013;**116**:336–47. https://doi.org/10.1016/j.zool.2013.08.005

Corn KA, Friedman ST. The rise of biting during the Cenozoic fueled reef fish body shape diversification. 2022;**119**: e21198281191–8.

Corn KA, Martinez CM, Burress ED *et al.* A multifunction trade-off has contrasting effects on the evolution of form and function. *Systematic Biology* 2021;**70**:681–93. https://doi.org/10.1093/sysbio/syaa091

Cox PG, Faulkes CG, Bennett NC. Masticatory musculature of the African mole-rats (Rodentia: Bathyergidae). *PeerJ* 2020;**8**:e8847. https://doi.org/10.7717/peerj.8847

Datovo A, Vari RP. The jaw adductor muscle complex in teleostean fishes: evolution, homologies and revised nomenclature (Osteichthyes: Actinopterygii). *PLoS One* 2013;**8**:e60846. https://doi.org/10.1371/journal.pone.0060846

Datovo A, Vari RP. The adductor mandibulae muscle complex in lower teleostean fishes (Osteichthyes: Actinopterygii): comparative anatomy, synonymy, and phylogenetic implications. *Zoological Journal of the Linnean Society* 2014;**171**:554–622. https://doi.org/10.1111/zoj.12142

Dickinson E, Stark H, Kupczik K. Non-destructive determination of muscle architectural variables through the use of DiceCT. *Anatomical Record (Hoboken, N.J.: 2007)* 2018;**301**:363–77. https://doi.org/10.1002/ar.23716

Dickinson E, Basham C, Rana A *et al.* Visualization and quantification of digitally dissected muscle fascicles in the masticatory muscles of *Callithrix jacchus* using nondestructive DiceCT. *Anatomical Record (Hoboken, N. J.: 2007)* 2019;**302**:1891–900. https://doi.org/10.1002/ar.24212

Elshoud-Oldenhave MJW. Prey capture in the pike-perch, *Stizostedion lucioperca* (Teleostei, Percidae). *Zoomorphologie* 1979;**93**:1–32. https://doi.org/10.1007/bf02568672

Elshoud-Oldenhave MJW, Osse JW. Functional morphology of the feeding system in the ruff—*Gymnocephalus cernua* (L. 1758)—(Teleostei, Percidae). *Journal of Morphology* 1976;**150**:399–422.

Etnier DA, Starnes WC. *The Fishes of Tennessee.* Knoxville: University of Tennessee Knoxville, 1993.

Friel JP, Wainwright PC. Evolution of complexity in motor patterns and jaw musculature of tetraodontiform fishes. *Journal of Experimental Biology* 1999;**202 (Pt 7)**:867–80. https://doi.org/10.1242/jeb.202.7.867

Gans C, de Vree F. Functional bases of fibre length and angulation in muscle. *Journal of Morphology* 1987;**192**:63–85. https://doi.org/10.1002/jmor.1051920106

Gignac PM, Kley NJ. The utility of DiceCT imaging for high-throughput comparative neuroanatomical studies. *Brain, Behavior and Evolution* 2018;**91**:180–90. https://doi.org/10.1159/000485476

Gignac PM, Kley NJ, Clarke JA *et al.* Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *Journal of Anatomy* 2016;**228**:889–909. https://doi.org/10.1111/joa.12449

Gobalet KW. Cranial specializations of parrotfishes, genus *Scarus* (Scarinae, Labridae) for scraping reef surfaces. In *Biology of Parrotfishes.* CRC Press, 2018:1–10.

Goodwin MR, Arbour JH. Darter fishes exhibit variable intraspecific head shape allometry and modularity. *Anatomical Record* 2022;**306**:446–56. https://doi.org/10.1002/ar.25088

Hanken J, Wake DB. Miniaturization of body size: organismal consequences and evolutionary significance. *Annual Review of Ecology and Systematics* 1993;**24**:501–19.

Harmon LJ, Schulte JA, Larson A *et al.* Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 2003;**301**:961–4. https://doi.org/10.1126/science.1084786

Harmon LJ, Weir JT, Brock CD *et al.* GEIGER: investigating evolutionary radiations. *Bioinformatics* 2008;**24**:129–31. https://doi.org/10.1093/bioinformatics/btm538

Herrel A, De Smet A, Aguirre LF *et al.* Morphological and mechanical determinants of bite force in bats: do muscles matter? *The Journal of Experimental Biology* 2008;**211**:86–91. https://doi.org/10.1242/jeb.012211

Hulsey CD, Mims MC, Streelman JT. Do constructional constraints influence cichlid craniofacial diversification? *Proceedings Biological Sciences* 2007;**274**:1867–75. https://doi.org/10.1098/rspb.2007.0444

Hulsey CD, Mims MC, Parnell NF *et al.* Comparative rates of lower jaw diversification in cichlid adaptive radiations. *Journal of Evolutionary Biology* 2010;**23**:1456–67. https://doi.org/10.1111/j.1420-9101.2010.02004.x

Hulsey CD, Holzman R, Meyer A. Dissecting a potential spandrel of adaptive radiation: Body depth and pectoral fin ecomorphology coevolve in Lake Malawi cichlid fishes. *Ecology and Evolution* 2018;**8**:11945–53. https://doi.org/10.1002/ece3.4651

Katzke J, Puchenkov P, Stark H *et al.* A roadmap to reconstructing muscle architecture from CT data. *Integrative Organismal Biology* 2022;**4**:obac001. https://doi.org/10.1093/iob/obac001

Khabbazian M, Kriebel R, Rohe K *et al.* Fast and accurate detection of evolutionary shifts in Ornstein–Uhlenbeck models. *Methods in Ecology and Evolution* 2016;**7**:811–24. https://doi.org/10.1111/2041-210x.12534

Kolmann MA, Huber DR, Dean MN *et al.* Myological variability in a decoupled skeletal system: Batoid cranial anatomy. *Journal of Morphology* 2014;**275**:862–81. https://doi.org/10.1002/jmor.20263

Kolmann MA, Nagesan RS, Andrews JV *et al.* DiceCT for fishes: recommendations for pairing iodine contrast agents with μCT to visualize soft tissues in fishes. *Journal of Fish Biology* 2023;**102**:893–903. https://doi.org/10.1111/jfb.15320

Konstantinidis P, Harris MP. Same but different: ontogeny and evolution of the *Musculus* adductor mandibulae in the Tetraodontiformes. *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 2011;**316B**:10–20. https://doi.org/10.1002/jez.b.21375

López-Fernández H, Arbour JH, Winemiller KOKO *et al.* Testing for ancient adaptive radiations in Neotropical cichlid fishes. *Evolution* 2013;**67**:no–no.

Lujan NK, Conway KW. Life in the fast lane: a review of rheophily in freshwater fishes. In: Riesch R, Tobler M, Plath M (eds), *Extremophile Fishes: Ecology, Evolution, and Physiology of Teleosts in Extreme Environments.* Switzerland: Springer International Publishing, 2015, 107–307.

Macrì S, Aalto IM, Allemand R *et al.* Reconstructing the origin and early evolution of the snake brain. *Science Advances* 2023;**9**:1–11.

Martin ML, Travouillon KJ, Fleming PA *et al.* Review of the methods used for calculating physiological cross-sectional area (PCSA) for ecological questions. *Journal of Morphology* 2020;**281**:778–89. https://doi.org/10.1002/jmor.21139

Muller M. A novel classification of planar four-bar linkages and its application to the mechanical analysis of animal systems. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 1996;**351**:689–720. https://doi.org/10.1098/rstb.1996.0065

O'Brien DM, Boisseau RP, Duell M *et al.* Muscle mass drives cost in sexually selected arthropod weapons. *Proceedings of the Royal Society B: Biological Sciences* 2019;**286**:20191063. https://doi.org/10.1098/rspb.2019.1063

de Oliveira L, Gower DJ, Wilkinson M *et al.* Comparative morphology of oral glands in snakes of the family Homalopsidae reveals substantial variation and additional independent origins of salt glands within Serpentes. *Journal of Anatomy* 2024;**244**:708–21. https://doi.org/10.1111/joa.14005

Orr JW. Feeding behavior of the greenbreast darter, *Etheostoma jordani* (Percidae: Etheostomatini). *Copeia* 1989;**1989**:202–6. https://doi.org/10.2307/1445626

Osse JW. Functional morphology of the head of the perch (*Perca fluviatilis* L.): an electromyographic study. *Netherlands Journal of Zoology* 1969;**19**:289–392. https://doi.org/10.1163/002829669x00134

Page L. *Handbook of Darters.* Neptune City, NJ: T.F.H. Publications Inc., 1983.

Paine MD, Dodson JJ, Power G. Habitat and food resource partitioning among four species of darters (Percidae, Etheostoma) in a southern Ontario stream. *Canadian Journal of Zoology* 1982;**60**:1635–41. https://doi.org/10.1139/z82-214

Revell LJ. Size-correction and principal components for interspecific comparative studies. *Evolution* 2009;**63**:3258–68. https://doi.org/10.1111/j.1558-5646.2009.00804.x

Revell LJ. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 2012;**3**:217–23. https://doi.org/10.1111/j.2041-210x.2011.00169.x

Santana SE. Quantifying the effect of gape and morphology on bite force: biomechanical modeling and in vivo measurements in bats. *Functional Ecology* 2016;**30**:557–65. https://doi.org/10.1111/1365-2435.12522

Santana SE. Comparative anatomy of bat jaw musculature via diffusible iodine-based contrast-enhanced computed tomography. *Anatomical Record (Hoboken, N.J.: 2007)* 2018;**301**:267–78. https://doi.org/10.1002/ar.23721

Santana SE, Arbour JH, Curtis A *et al.* 3D digitization in functional morphology: where is the point of diminishing returns? *Integrative and Comparative Biology* 2019;**59**:1–13.

Schroth N. Evolution of color and pattern in etheostomatinae (Darter Fishes). *MS thesis.* Middle Tennessee State University, 2024.

Slater GJ, Pennell MW. Robust regression and posterior predictive simulation increase power to detect early bursts of trait evolution. *Systematic Biology* 2014;**63**:293–308. https://doi.org/10.1093/sysbio/syt066

Slater GJ, Price SA, Santini F *et al.* Diversity versus disparity and the radiation of modern cetaceans. *Proceedings of the Royal Society B* 2010a;**277**:3097–104. https://doi.org/10.1098/rspb.2010.0408

Slater GJ, Price S, Santini F *et al.* Diversity versus disparity and the radiation of modern cetaceans. *Proceedings of the Royal Society B* 2010b;**277**:3097–104. https://doi.org/10.1098/rspb.2010.0408

Somjee U. Positive allometry of sexually selected traits: do metabolic maintenance costs play an important role? *Bioessays* 2021;**43**:1–13.

Sullivan SP, McGechie FR, Middleton KM *et al.* 3D muscle architecture of the pectoral muscles of European starling (*Sturnus vulgaris*). *Integrative Organismal Biology* 2019;**1**:1–18.

Turingan RG. Ecomorphological relationships among Caribbean tetraodontiform fishes. *Journal of Zoology* 1994;**233**:493–521. https://doi.org/10.1111/j.1469-7998.1994.tb05279.x

Turner CL. Food of the common Ohio darters. *Ohio Journal of Science* 1921;**22**:41–62.

Wainwright PC, Richard BA. Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes* 1995;**44**:97–113. https://doi.org/10.1007/bf00005909

Wainwright PC, Ferry-Graham LA, Waltzek TB *et al.* Evaluating the use of ram and suction during prey capture by cichlid fishes. *The Journal of Experimental Biology* 2001;**204**:3039–51. https://doi.org/10.1242/jeb.204.17.3039

Wainwright PC, Bellwood DR, Westneat MW *et al*. A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. *Biological Journal of the Linnean Society* 2004;**82**:1–25. https://doi.org/10.1111/j.1095-8312.2004.00313.x

Weitzman S, Vari R. Miniaturization in South American freshwater fishes: an overview and discussion. *Proceedings of the Biological Society of Washington* 1988;**101**:444–65.

Westneat MW. Feeding mechanics of teleost fishes (Labridae; Perciformes): a test of four-bar linkage models. *Journal of Morphology* 1990;**205**:269–95. https://doi.org/10.1002/jmor.1052050304

Westneat MW. Transmission of force and velocity in the feeding mechanisms of labrid fishes (Teleostei, Perciformes). *Zoomorphology* 1994;**114**:103–18. https://doi.org/10.1007/bf00396643

Westneat MW. A biomechanical model for analysis of muscle force, power output and lower jaw motion in fishes. *Journal of Theoretical Biology* 2003;**223**:269–81. https://doi.org/10.1016/s0022-5193(03)00058-4

Widrig KE, Bhullar BAS, Field DJ. 3D atlas of tinamou (Neornithes: Tinamidae) pectoral morphology: Implications for reconstructing the ancestral neornithine flight apparatus. *Journal of Anatomy* 2023;**243**:729–57. https://doi.org/10.1111/joa.13919