



Fish scale shape follows predictable patterns of variation based on water column position, body size, and phylogeny

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Abstract

The influence of environment and phylogeny on morphological characteristics of organisms is well documented. However, little is known about how these factors influence scale shape in fishes, a feature which may be important for drag reduction. We evaluated the impact of both on scale shape variation in the primarily benthic, riverine darter clade (Percidae: Etheostomatinae) of fishes. We predicted that darters with close phylogenetic relationships and/or shared ecologies would have more similar scale shapes, but this relationship would be mediated by use of the substrate boundary layer. We used geometric morphometrics and seven homologous landmarks for 92 species of darters representing all genera and 37 clades within genera to measure scale shape. Phylogenetic relationships and ecological variables describing habitat, spawning mode, and maximum body size of each species were summarized from the literature. We used ordinations to examine scale shape variation among phylogenetic and ecological groups. We conducted Phylogenetic Generalized Least Squares analyses to test for relationships between scale shape and ecological characteristics. Scale shape variation occurred within and among darter clades, and was significantly related to phylogeny. However, we found divergent scale shapes between close relatives and similar scale shapes between distantly related species. After accounting for phylogenetic signal, size and water column position were related to scale shape. Extra-large, hyperbenthic species had longer, narrower scales that may decrease laminar drag. Sub-benthic darters had scales that were narrower at the insertion, and with enlarged ventral margins that may facilitate burying. Among benthic darters, size was significantly related to scale shape though a lack of clustering among many taxonomic and ecological groups may indicate that boundary layer use has reduced selective pressures from drag. Our results are consistent with others that have found both environment and phylogeny influence Teleost fish morphology.

Keywords Benthic · Boundary layer · Darter · Ecomorphology · Flow · Geometric morphometrics

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Introduction

Evolutionary history, or phylogeny, imposes constraints on morphology, physiology, and behavioral phenotypes across the Tree of Life (Guill et al. 2003; Rodríguez-González et al. 2017; Ospina-Garcés et al. 2018). Environment and niche space can also constrain trait diversity (Losos et al. 1998; Zelditch et al. 2017; Watanabe et al. 2019) and may frequently contribute to convergence of traits among distantly related taxa (Losos 1990; Agrawal 2017; Pigot et al. 2020). Trait convergence is common among species that share environments that impose strong physical constraints on organisms, such as aquatic environments (McGhee 2011; Bower et al. 2021). For example, many distantly related fishes (teleosts and sharks) and aquatic mammals have fusiform or streamlined bodies, an adaptation that mitigates the physical constraints of hydrodynamic drag and water viscosity on locomotion, leading to improved swimming performance (Webb 1984; Tavera et al. 2018; Velotta et al. 2018; Burns and Sidlauskas 2019).

In addition to water viscosity, flow velocity and type (e.g., fast vs. slow and turbulent vs. laminar flows) may impose strong and predictable selective forces on fish morphology (Langerhans 2008; Wainwright 2019; Rincon-Sandoval 2020; Bower et al. 2021). For example, fishes living in low or unpredictable flow environments (ponds, lakes, pools or shallow riffles of streams, or tidal zones) have fin and body shapes that maximize unsteady-state (highly maneuverable) swimming, while those living in fast and/or unidirectional flow environments have fin and body shape traits that maximize steady-state (highly streamlined) swimming (Brinsmead and Fox 2002; Langerhans 2008; Foster et al. 2015; Wainwright 2019). Fishes occupying extreme high-flow environments often have unique morphological adaptations for station-holding such as suckers, enlarged pectoral fins, and pectoral spines (Casatti and Castro 2006; Leal et al. 2011; Pagotto et al. 2011). However, the degree to which flow influences morphology can be limited by phylogenetic constraint (Krabbenhof et al. 2009; Foster et al. 2015). Additionally, aspects of fish behavior in low versus high-flow environments can reverse the link between steady/unsteady-state morphologies and local flow regimes (Krabbenhof et al. 2009; Meyers and Belk 2014; Bower and Piller 2015).

Water column position (benthic vs. pelagic) is another important selective force that impacts the shape of aquatic organisms. Recent studies found strong evolutionary determinism in fish morphology associated with water column position and the transition between occupying benthic and pelagic environments (Rincon-Sandoval et al. 2020). After invasion of pelagic environments from benthic lineages, deep-bodied, benthic fishes with truncate caudal fins repeatedly gave rise to slender-bodied, pelagic fishes with furcate caudal fins (Rincon-Sandoval et al. 2020). In general, body elongation is a common trait among lineages that have invaded and diversified within pelagic environments (Claverie and Wainwright 2014; Burrell et al. 2017; Tavera et al. 2018). Variation in scale surface topography (roughness or rugosity) has also been linked to benthic and pelagic water column positions in damselfishes, suggesting that the divergent flow types—laminar (pelagic) versus turbulent (benthic)—may impact scale traits (Wainwright 2019).

Despite clear influences of flow type, velocity, and water column position on fish morphology, the effects of such environmental influences may be overall less predictable for benthic riverine fishes, than for those living in the water column, or those in lentic environments (Langerhans 2008). Many benthic riverine fishes seek shelter in the substrate boundary layer, where they experience reduced flow across the body, even in high flow habitats such as stream riffles where flow type is more likely to be turbulent due to the flow of water

across various sized substrates. Use of the boundary layer likely contributes to reduction in the expected selective pressures of flow on morphological traits in these fishes (Page and Swofford 1984; Langerhans 2008; Carlson and Lauder 2011).

With over 250 described species, darters (Percidae: Etheostomatinae) are an ideal clade to examine relationships between phylogeny, benthic versus non-benthic environmental factors, and morphology. These primarily benthic fishes are known for their flashy breeding colors and darting movements on the bottom of streams and are diverse in habitat requirements and morphologies (Kuehne and Barbour 1983; Page 1983; Carlson and Wainwright 2010; Geheber and Frenette 2016). In general, body morphology and ecology are described as conserved within clades of darters (Ciccotto and Mendelson 2015; Guill et al. 2003; Geheber and Frenette 2016). However, in some cases sister species are highly divergent in both body morphology and ecology (Geheber and Frenette 2016). Variations in darter morphology are related to breeding behaviors, habitat, and prey acquisition strategies (Paine et al. 1982; Page and Swofford 1984; Guill et al. 2003; Carlson and Wainwright 2010; Martin and Page 2015), but in several examples, environmental factors contribute to morphological convergence (Page and Swofford 1984; Guill et al. 2003; Carlson and Wainwright 2010; Geheber and Frenette 2016).

Although both phylogeny and environment (particularly flow) are known to influence darter phenotypes, few studies have examined the effect of these forces on scale shape (Coburn and Gaglione 1992). Fish scale traits have a long history of use in taxonomic and systematic studies, and other works have found that the morphology of fish scales can be used to classify individuals to the species- or even population-level (Ibáñez et al. 2007, 2009; Renjith et al. 2014). Investigations of fish ecomorphology and trait convergence have focused primarily on body and fin shape (Langerhans 2008; Binning and Roche 2015; Oliveira 2021). The relationship between Teleost fish scales and the environment are less well known. However, like placoid scales in sharks, elasmoid scales of Teleost fishes may function to reduce drag (Oeffner and Lauder 2012). Therefore, fish scales may show similar trends in evolutionary adaptations to different flow types and water column positions as body shape and fin features. Accordingly, we evaluated the relative influence of phylogeny and environmental factors on darter scale shape variation. We hypothesized that scale shape variation was influenced jointly by phylogeny and flow. Flow experienced by fishes may be determined by factors including water column position, maximum body size, environment type, microhabitat, substrate type, spawning mode, and others (Page and Swofford 1984; Carlson and Wainwright 2010; Bossu and Near 2015; Geheber and Frenette 2016). Therefore, we predicted that darters with close phylogenetic relationships and/or similar characteristics determining flow experienced would have more similar scale shapes than more distantly related and/or dissimilar species in environmental variables. We also predicted that the relationship between scale shape and environmental variables would be weaker among darters that regularly utilize the boundary layer of streams.

Materials and methods

Species and ecological variable selection

To capture the potential range of scale shape variation among darters, we selected at least one species from each terminal clade within each of the five recognized darter genera within Etheostomatinae (37 clades, 92 species total; Table 1; “all darter” dataset),

Table 1 Species used to assess scale shape variation in darters

Code	Species (# specimens)	Clade	Water column position	Environment	Microhabitat	Spawning mode	SL (mm)	Substrate
01	<i>A. beani</i> (30)	<i>Ammocrypta</i>	Sub-benthic ¹	Lotic ^{1,2,3}	Run	Burier ⁴	51	Fine ⁵
02	<i>A. pellucida</i> (30)	<i>Ammocrypta</i>	Sub-benthic ¹	Lotic ^{1,5,6}	Run	Burier ⁴	46	Fine ⁵
03	<i>A. vivax</i> (30)	<i>Ammocrypta</i>	Sub-benthic ¹	Lotic ^{1,2,3}	Run	Burier ^{7,8}	49	Fine ⁵
04	<i>C. asprella</i> (30)	<i>Crystallaria</i>	Sub-benthic ¹	Lotic ^{3,6,9}	Run	Burier ⁴	88	Fine ⁵
05	<i>E. asprigene</i> (30)	<i>Astatichthys</i>	Benthic ¹⁰	General ^{3,5,6}	General	Attacher ^{4,11}	41	Coarse ⁵
06	<i>E. autumnale</i> (30)	<i>Psychromaster</i>	Benthic ¹⁰	Lotic ^{5,9}	Riffle	Burier ⁹	58	Coarse ⁵
07	<i>E. baileyi</i> (30)	<i>Ebaileyi-hisirio</i>	Benthic ¹⁰	Lotic ^{5,6,12}	General	Attacher ^{4,13,14}	39	Coarse ⁵
08	<i>E. barboursi</i> (30)	<i>Oopareia</i>	Benthic ¹⁰	Lotic ^{5,6,12}	Pool	Clusterer ^{4,12,15}	36	Coarse ⁵
09	<i>E. barrenense</i> (30)	<i>Ulocentra</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Attacher ^{1,2,14,16}	41	Coarse ⁵
10	<i>E. bison</i> (30)	<i>Ceasia</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Burier ¹²	42	Coarse ⁵
11	<i>E. blennius</i> (29)	<i>Neotheostoma</i>	Benthic ¹⁰	Lotic ^{2,5,6}	Riffle	Attacher ⁴	52	Coarse ⁵
12	<i>E. boschungii</i> (29)	<i>Psychromaster</i>	Benthic ¹⁰	Lotic ^{2,5,6}	Pool	Attacher ^{2,5,17}	45	Coarse ⁵
13	<i>E. brevispinum</i> (30)	<i>Richiella</i>	Benthic ¹⁰	Lotic ^{5,18}	Riffle	Clusterer ¹⁸	50	Coarse ⁵
14	<i>E. caeruleum</i> (26)	<i>Astatichthys</i>	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Burier ^{6,16,19}	48	Coarse ⁵
15	<i>E. chlorosoma</i> (29)	<i>Vaillantia</i>	Benthic ¹⁰	Lotic ^{3,5,6}	Pool	Burier ^{3,20}	42	Fine ⁵
16	<i>E. collis</i> (30)	<i>Ecollis</i>	Benthic ¹⁰	General ^{5,18,21}	Pool	Attacher ^{4,22}	36	Fine ⁵
17	<i>E. colorosum</i> (30)	<i>Adonia</i>	Benthic ¹⁰	Lotic ^{2,5}	Pool	Attacher ^{2,23}	42	Fine ⁵
18	<i>E. cragini</i> (30)	<i>Psychromaster</i>	Benthic ¹⁰	Lotic ^{5,6,9}	Pool	Burier ^{5,6,24}	39	Fine ⁵
19	<i>E. crossopterum</i> (28)	<i>Stigmacerca</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Clusterer ^{4,25}	52	Coarse ⁵
20	<i>E. derivativum</i> (30)	<i>Oopareia</i>	Benthic ¹⁰	Lotic ^{5,12}	Pool	Clusterer ^{1,2,15,25}	43	Coarse ⁵
21	<i>E. duryi</i> (30)	<i>Adonia</i>	Benthic ¹⁰	Lotic ¹²	General	Attacher ^{1,2,20,26}	43	Coarse ⁵
22	<i>E. edwini</i> (30)	<i>Eedwini</i>	Benthic ¹⁰	Lotic ^{2,5,27}	Run	Attacher ^{2,6,27}	33	Fine ⁵
23	<i>E. emieri</i> (30)	<i>Adonia</i>	Benthic ¹⁰	Lotic ¹²	General	Attacher ^{4,12,13}	46	Fine ⁵
24	<i>E. flavum</i> (29)	<i>Adonia</i>	Benthic ¹⁰	Lotic ¹²	General	Attacher ^{4,26,28}	49	Coarse ⁵
25	<i>E. fusiforme</i> (30)	<i>Hololepis</i>	Benthic ¹⁰	General ^{5,6,27}	Pool	Attacher ^{1,6,29}	35	Fine ⁵
26	<i>E. gracile</i> (27)	<i>Egracile</i>	Benthic ¹⁰	General ^{5,6,27}	Pool	Attacher ^{30,4,3}	41	Fine ⁵
27	<i>E. guselli</i> (30)	<i>Neotheostoma</i>	Benthic ¹⁰	Lotic ^{5,12}	Riffle	Attacher ⁴	82	Coarse ⁵

Table 1 (continued)

Code	Species (# specimens)	Clade	Water column position	Environment	Microhabitat	Spawning mode	SL (mm)	Substrate
28	<i>E. histrio</i> (30)	<i>Ebaileyi-histrio</i>	Benthic ¹⁰	Lotic ^{2,3,6}	Riffle	Attacher ^{4,14}	45	Fine ⁵
29	<i>E. jessiae</i> (29)	<i>Doration</i>	Benthic ¹⁰	Lotic ^{2,5,6}	General	Burter ^{1,12,31}	47	Coarse ⁵
30	<i>E. kanawhae</i> (30)	<i>Pocillichthys</i>	Benthic ¹⁰	Lotic ^{5,6,21}	General	Burter ⁴	54	Coarse ⁵
31	<i>E. kenicotti</i> (30)	<i>Richiella</i>	Benthic ¹⁰	Lotic ^{5,6,12}	Pool	Clusterer ^{3,23,34}	49	Coarse ⁵
32	<i>E. lachneri</i> (30)	<i>Adonia</i>	Benthic ¹⁰	Lotic ^{2,5}	Pool	Attacher ⁴	41	Fine ⁵
33	<i>E. lawrencei</i> (30)	<i>Ceasia</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Burter ¹²	40	Coarse ⁵
34	<i>E. lepidum</i> (29)	<i>Astatchthys</i>	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Attacher ^{5,12,35}	39	Coarse ⁵
35	<i>E. luteovinctum</i> (30)	<i>Astatchthys</i>	Benthic ¹⁰	General ^{5,12}	General	Burter ^{3,6}	46	Coarse ⁵
36	<i>E. lynceum</i> (30)	<i>Neotheostoma</i>	Benthic ¹⁰	Lotic ^{5,12}	Riffle	Attacher ⁴	45	Coarse ⁵
37	<i>Ai. maydeni</i> (22)	<i>Amydeni</i>	Benthic ¹⁰	Lotic ^{5,12,37}	Pool	Attacher ^{4,21}	68	Coarse ⁵
38	<i>E. microporca</i> (30)	<i>Microperca</i>	Benthic ¹⁰	Lotic ^{5,6,38}	Pool	Attacher ^{1,6,19,38}	27	Fine ⁵
39	<i>E. nigrum</i> (30)	<i>Boleosoma</i>	Benthic ¹⁰	General ¹²	Run	Clusterer ^{1,16,19}	49	Fine ⁵
40	<i>E. nuchale</i> (23)	<i>Astatchthys</i>	Benthic ¹⁰	General ^{2,5,6}	Pool	Attacher ^{3,9}	37	Fine ⁵
41	<i>E. okaloosae</i> (30)	<i>Eokaloosae</i>	Benthic ¹⁰	Lotic ^{5,6,27}	Run	Attacher ^{4,6,40}	31	Fine ⁵
42	<i>E. oophylax</i> (29)	<i>Stigmacerca</i>	Benthic ¹⁰	Lotic ^{5,12}	Pool	Clusterer ^{4,12,25}	46	Fine ⁵
43	<i>E. parvipinne</i> (30)	<i>Fuscatelum</i>	Benthic ¹⁰	Lotic ^{2,3,5}	Pool	Attacher ^{2,3,41}	38	Fine ⁵
44	<i>E. planasaxatile</i> (29)	<i>Ulocentra</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Attacher ¹²	44	Coarse ⁵
45	<i>E. podostenone</i> (30)	<i>Boleosoma</i>	Benthic ¹⁰	Lotic ^{5,6,21}	General	Clusterer ^{4,21,42}	43	Coarse ⁵
46	<i>E. proeliare</i> (29)	<i>Microperca</i>	Benthic ¹⁰	General ^{1,5,6}	Pool	Attacher ^{3,6,43}	34	Fine ⁵
47	<i>E. pseudovulatum</i> (32)	<i>Stigmacerca</i>	Benthic ¹⁰	Lotic ^{5,12}	Pool	Clusterer ¹²	50	Fine ⁵
48	<i>E. pyrrhogaster</i> (30)	<i>Adonia</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Attacher ^{4,12,44}	41	Fine ⁵
49	<i>E. radiosum</i> (30)	<i>Vexillapinna</i>	Benthic ¹⁰	General ^{5,6,9}	General	Burter ^{6,9,45}	50	Coarse ⁵
50	<i>E. sagitta</i> (30)	<i>Litocara</i>	Benthic ¹⁰	Lotic ^{5,6,12}	General	Burter ^{4,12,44}	57	Coarse ⁵
51	<i>E. sitikuense</i> (30)	<i>Richiella</i>	Benthic ¹⁰	Lotic ^{5,12}	General	Clusterer ¹²	53	Coarse ⁵
52	<i>E. smithi</i> (30)	<i>Oopareia</i>	Benthic ¹⁰	General ^{5,6,12}	Pool	Clusterer ^{4,12,34}	41	Coarse ⁵

Table 1 (continued)

Code	Species (# specimens)	Clade	Water column position	Environment	Microhabitat	Spawning mode	SL (mm)	Substrate
53	<i>E. spectabile</i> (30)	Ceasia	Benthic ¹⁰	Lotic ^{9,38}	General	Burier ^{9,22,46}	46	Coarse ⁵
54	<i>E. stigmaeum</i> (30)	Doration	Benthic ¹⁰	Lotic ^{2,5,6}	General	Burier ^{1,2,31}	42	Fine ⁵
55	<i>E. swaini</i> (30)	Astactichthys	Benthic ¹⁰	Lotic ^{1,3,6}	Pool	Burier ^{1,3,47}	42	Coarse ⁵
56	<i>E. swamanoa</i> (30)	Neotheostoma	Benthic ¹⁰	Lotic ^{5,6,12}	General	Attacher ^{4,8}	56	Coarse ⁵
57	<i>E. tetrazonum</i> (30)	Poecilichthys	Benthic ¹⁰	Lotic ^{5,38}	Riffle	Burier ^{1,4,38}	57	Coarse ⁵
58	<i>E. variatum</i> (30)	Poecilichthys	Benthic ¹⁰	Lotic ^{5,21,48}	Riffle	Burier ^{4,6,8}	59	Coarse ⁵
59	<i>E. vitreum</i> (30)	Boleosoma	Sub-benthic ¹	Lotic ^{5,21,49}	General	Clusterer ^{4,49}	47	Fine ⁵
60	<i>E. whipplei</i> (30)	Vexillapinna	Benthic ¹⁰	Lotic ^{5,6,9}	General	Burier ⁴	46	Coarse ⁵
61	<i>E. zonale</i> (30)	Neotheostoma	Benthic ¹⁰	Lotic ^{5,12}	Riffle	Attacher ^{1,2,38,50}	46	Coarse ⁵
62	<i>E. zonisitium</i> (30)	Adonia	Benthic ¹⁰	Lotic ^{1,12}	General	Attacher ^{4,12,44}	40	Fine ⁵
63	<i>N. acuticeps</i> (30)	Nothonotus	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Burier ^{4,12}	53	Coarse ⁵
64	<i>N. bellus</i> (27)	Nothonotus	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Burier ^{4,51}	50	Coarse ⁵
65	<i>N. camurus</i> (28)	Nothonotus	Benthic ¹⁰	Lotic ^{1,6,12}	Riffle	Burier ^{4,6,12}	49	Coarse ⁵
66	<i>N. jordani</i> (30)	Nothonotus	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Burier ^{4,12,52}	46	Coarse ⁵
67	<i>N. microlepidus</i> (27)	Nothonotus	Benthic ¹⁰	Lotic ^{1,6,12}	Riffle	Clusterer ^{4,12,20}	56	Coarse ⁵
68	<i>N. rufilineatus</i> (28)	Nothonotus	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Burier ^{4,12,53}	46	Coarse ⁵
69	<i>N. tippecanoe</i> (30)	Nothonotus	Benthic ¹⁰	Lotic ^{5,6,12}	Riffle	Burier ^{4,12,54}	27	Coarse ⁵
70	<i>P. aurantiaca</i> (30)	Paurantiaca	Hyperbenthic ¹⁰	Lotic ^{5,6,12}	General	Burier ^{4,6,12}	86	Coarse ⁵
71	<i>P. caprodes</i> (30)	Pileoma	Hyperbenthic ¹⁰	General ^{1,6,9}	General	Burier ^{4,9}	102	Fine ⁵
72	<i>P. copelandi</i> (29)	Cottogaster	Benthic ¹⁰	General ^{4,6,9}	General	Burier ^{4,6,9}	46	Fine ⁵
73	<i>P. crassa</i> (29)	Atlantis	Benthic ¹⁰	Lotic ^{1,5,6}	General	Burier ⁴	48	Coarse ⁵
74	<i>P. evides</i> (26)	Pevides	Benthic ¹⁰	Lotic ^{3,5,12}	General	Burier ^{3,4}	51	Coarse ⁵
75	<i>P. gymnocephala</i> (31)	Alvordius	Hyperbenthic ¹⁰	Lotic ^{5,21}	Riffle	Burier ⁴	56	Coarse ⁵
76	<i>P. kathae</i> (30)	Pileoma	Hyperbenthic ¹⁰	Lotic ^{5,12}	General	Burier ⁴	105	Coarse ⁵
77	<i>P. lentacula</i> (30)	Hadropterus	Hyperbenthic ¹⁰	Lotic ^{2,3,5}	Riffle	Burier ⁴	121	Coarse ⁵

Table 1 (continued)

Code	Species (# specimens)	Clade	Water column position	Environment	Microhabitat	Spawning mode	SL (mm)	Substrate
78	<i>P. macrocephala</i> (13)	<i>Pagella</i>	Hyperbenthic ¹⁰	Lotic ^{5,6,12}	Pool	Burier ⁴	83	Coarse ⁵
79	<i>P. macrolepida</i> (30)	<i>Pileoma</i>	Hyperbenthic ¹⁰	General ^{5,6}	General	Burier ⁴	82	Fine ⁵
80	<i>P. maculata</i> (30)	<i>Alvordius</i>	Hyperbenthic ¹⁰	Lotic ^{5,55}	Pool	Burier ^{4,56}	56	Fine ⁵
81	<i>P. neviseuse</i> (30)	<i>Atlantis</i>	Benthic ¹⁰	Lotic ^{1,5,6}	General	Burier ^{6,21}	69	Fine ⁵
82	<i>P. nigrofasciata</i> (30)	<i>Hadropterus</i>	Hyperbenthic ¹⁰	Lotic ^{3,5,6}	Pool	Burier ⁴	59	Fine ⁵
83	<i>P. palmaris</i> (30)	<i>Hadropterus</i>	Benthic ¹⁰	Lotic ^{1,2,12}	Riffle	Burier ⁴	64	Coarse ⁵
84	<i>P. phoxocephala</i> (30)	<i>Swainia</i>	Hyperbenthic ¹⁰	Lotic ^{5,6,55}	Riffle	Burier ^{4,57}	56	Coarse ⁵
85	<i>P. rex</i> (19)	<i>Pileoma</i>	Hyperbenthic ¹⁰	Lotic ^{5,6,21}	General	Burier ²¹	83	Coarse ⁵
86	<i>P. roanoka</i> (30)	<i>Atlantis</i>	Benthic ¹⁰	Lotic ^{5,6,21}	Riffle	Burier ^{4,21}	46	Coarse ⁵
87	<i>P. sciera</i> (30)	<i>Hadropterus</i>	Hyperbenthic ¹⁰	Lotic ^{1,5,6}	General	Burier ⁴	61	Coarse ⁵
88	<i>P. shumardi</i> (30)	<i>Imostoma</i>	Benthic ¹⁰	Lotic ^{3,5,6}	Riffle	Burier ⁴	60	Coarse ⁵
89	<i>P. smithvanizi</i> (28)	<i>Hadropterus</i>	Hyperbenthic ¹⁰	Lotic ^{2,5}	General	Burier ⁴	45	Coarse ⁵
90	<i>P. squamata</i> (30)	<i>Swainia</i>	Hyperbenthic ¹⁰	Lotic ^{5,6,12}	General	Burier ⁴	92	Coarse ⁵
91	<i>P. stictogaster</i> (30)	<i>Odontopholis</i>	Hyperbenthic ¹⁰	Lotic ^{5,6,12}	Pool	Burier ^{4,58}	51	Fine ⁵
92	<i>P. vigil</i> (30)	<i>Imostoma</i>	Benthic ¹⁰	Lotic ^{3,5}	General	Burier ⁴	51	Fine ⁵

The number of individuals examined for each species is in parentheses and genus abbreviations are: “A” = *Ammocrypta*, “C” = *Crystallaria*, “E” = *Etheostoma*, “A1” = *Allothitium*, “N” = *Nothotus*, and “P” = *Percina*. Classification and clade designations follow Near et al. (2011) with the addition of genus *Allothitium* following MacGuigan and Near (2018). Code labels are those used to reference species throughout the text and in Figs. 1, 2, 3 and 4. Categorical ecological variable assignments used for each species were taken from literature. Standard length in mm (size) was recorded for each specimen and averaged within species. Superscript numbers correspond to numbered citations in the footnote which are fully cited in the References section of the text

1. Page (1983); 2. Boshung and Mayden (2004); 3. Ross and Brenneman (2001); 4. Page et al. (1985); 5. Page and Burr (2011); 6. Kuehne and Barbour (1983); 7. Muller (2008); 8. Simon and Wallus (2006); 9. Robison and Buchanan (1988); 10. Bossu and Near (2015); 11. Cummings et al. (1984); 12. Eimier and Starnes (1993); 13. Porterfield (1998); 14. Steinberg et al. (2000); 15. Page et al. (1992); 16. Winn (1958a); 17. Boshung and Nieland (1986); 18. Rohde et al. (2009); 19. Winn (1958b); 20. Page et al. (1983); 21. Jenkins and Burkhead (1993); 22. Kelly et al. (2012); 23. Johnston et al. (1999); 24. Distler (1972); 25. Page (2000); 26. Porterfield (1997); 27. Robins et al. (2018); 28. Keevin et al. (1989); 29. Fletcher (1976); 30. Braasch and Smith (1967); 31. Simon (1997); 32. Page (1975); 33. Page (1976); 34. Page and Burr (1976); 35. Strawn (1956); 36. Johnston and Johnson (2000); 37. Compton and Taylor (2013); 38. Pflieger et al. (1997); 39. Duncan et al. (2010); 40. Collette and Yeger (1962); 41. Johnston (1994); 42. Jenkins (1980); 43. Burr and Page (1978); 44. Carney and Burr (1989); 45. Scalet (1973); 46. Mendelson (2003); 47. Ruple et al. (1984); 48. Trautman (1981); 49. Winn and Picciolo (1960); 50. Walters (1994); 51. Fisher (1990); 52. Orr and Ramsey (1990); 53. Widlak and Neves (1985); 54. Warren et al. (1986); 55. Smith (1979); 56. Petravicz (1938); 57. Page and Smith (1971); 58. Burr and Page (1993)

following the phylogeny of Near et al. (2011). However, we labelled the previously unresolved *Etheostoma maydeni* as *Allohistium maydeni*, per the recommendation of MacGuigan and Near (2018). To more explicitly examine the effects of environmental variables such as substrate size and flow experienced on scale shape variation in a benthic environment, we trimmed the all darter dataset to one that included only those darter species that were classified as benthic in water column position (Bossu and Near 2015; 72 species total; Table 1; “benthic darter” dataset).

We assessed the influence of ecological characteristics on scale shape by summarizing traits including water column position (WCP; benthic, hyperbenthic, sub-benthic; for the all species dataset only), environment type (lotic vs. generalist), microhabitat type (riffle, run, pool), spawning mode (sensu Page 1983), substrate size (fine vs. coarse), and body size (size) of specimens averaged within species (measurements in Table 1). For WCP we classified species as benthic (those primarily living on the substrate) or hyperbenthic (living primarily above the substrate) based on Bossu and Near (2015), but added a sub-benthic category for species that bury frequently in the substrate outside the spawning season (Kuehne and Barbour 1983; Page 1983; Page and Burr 2011). We classified darters as either lotic specialists or generalists in environment type (Table 1). Because we sampled only three lentic specialists (*E. fusiforme*, *E. proeliare*, *E. nuchale*), we grouped them with the generalists, since they likely experience flows more similar to generalists than to lotic specialists. We summarized microhabitat categories into “pool” (slower or non-flowing, deeper waters), “run” (moderate flow and depth), “riffle” (faster flowing, shallow waters) (Page and Burr 2011), or “generalists” (species which frequent multiple microhabitat types; Table 1). We included four spawning modes recognized by Page (1983): egg “attachers”, “buriers”, “clusterers”, and “clumpers” (Table 1). Darters classified as “attachers” adhere eggs to aquatic structures like substrate, logs, and submerged vegetation; “buriers” dig themselves into the substrate so eggs are released into the interstitial spaces; “clusterers” flip upside down under slabrocks and lay eggs closely in a single layer, and “clumpers” wedge their bodies into the interface between a boulder and the underlying substrate to group their eggs in the crevice. We were only able to sample one species from the clumping category (*Nothonotus microlepidus*), which we placed into the clusterer group for analyses. We used two substrate type classifications based on Page and Burr (2011) including “Fine” and “Coarse” categories. These serve as a proxy for the relative size of the boundary layer that is potentially available to a darter, and the type of flows they will encounter outside of this shelter. Fine substrates (bedrock, sand, and silt) are presumed to have little boundary layer and more laminar flows, and coarse substrates (rock, gravel, or cobble) are presumed to have at least some amount of accessible boundary layer and more turbulent flows (Carlson and Lauder 2011).

The standard length (SL) of each specimen was recorded to the nearest mm and averaged within each species. Though size was analyzed as a continuous variable, for ease of visualization in graphs we assigned species to size groups created to include equal cumulative percentages of averaged body sizes from a frequency distribution. For the all species dataset, we considered a species “extra-large” if maximum body size was between 56 and 121 mm standard length (SL), “large” if maximum body size fell at or between 47 and 54 mm SL, “medium” from 42 to 46 mm SL, and “small” from 27 to 41 mm SL (Table 1). In the benthic species dataset, darters were considered “small” if standard length was at or between 27 and 40 mm, “medium” from 41 to 45 mm, “large” from 46 to 49 mm and “extra-large” from 50 to 82 mm. In rare instances, classification information for an ecological variable was not readily available at the species level (due to the recent rise in the

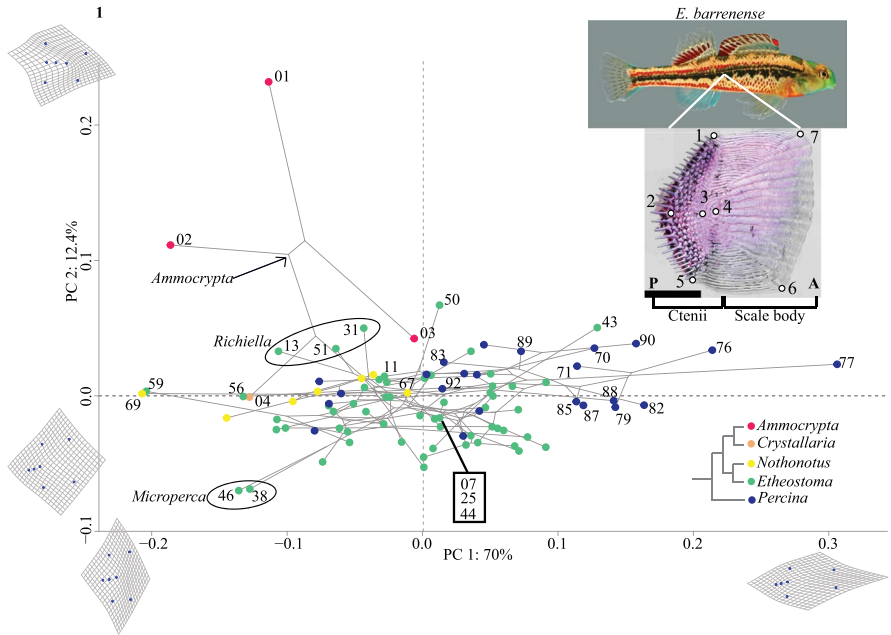


Fig. 1 Principal component analysis (PCA) of average scale shape for all 92 Etheostomatinae darter species examined. Dots on the graph represent the averaged scale shape score for each species and are colored by genus-level clades. Numbers identify species codes and correspond to those in Table 1. Transformation grids on Principal Component axes 1 and 2 depict scale shape changes relative to the overall average scale shape of all darter species along each respective axis. Lines connecting points represent the phylogenetic relationships among species based on Near et al. (2011). The inset phylogeny at the bottom right of the figure shows relationships among darter genera (Near et al. 2011). The upper right image of *E. barrenense* (photo credit: Mark Hoger) shows the body placement from which scales were extracted from all specimens and an enlarged scale image that shows the placement of the 7 landmarks (white dots) used to assess scale shape variation. Letters “A” and “P” on the scale image denote “anterior” and “posterior” regions of the scale. Clades highlighted in the text are labelled and circled or have an arrow pointing to the clade node

descriptions of darter species complexes), and in those cases we estimated values based on data published for its sister taxon (Table 1).

Specimen and scale selection

Using museum specimens (Table 1 and Appendix 1), we removed one scale from the right side of each specimen, directly above the anal fin origin, one row below the lateral line (Fig. 1). This was the only area for all darter species with scales present near a fin origin, controlling for effects of scale location on analysis of shape (Ibáñez et al. 2009; Wainwright and Lauder 2016; Bräger et al. 2017). For most of the 92 species sampled, we extracted scales from 30 adult individuals to minimize potential effects of ontogenetic growth on scale shape data (Table 1 and Appendix 1). We ensured the use of adult specimens by selecting individuals that were equal to- or greater than the average adult standard length for each species based on published size data, or selected

sexually mature individuals (i.e., gravid females or males with breeding colors or characteristics including tubercles on fins or body, thickened skin on fins, head, or body, soft knobs on fins, or enlarged genital papilla; Page 1983). For each species we attempted to examine only individuals from the same location or river system to avoid confounding factors related to intraspecific variation and occurrence of cryptic species (Hopper et al. 2017; Appendix 1).

Shape data

After extracting each scale, we removed all soft tissue to enhance consistent placement of landmarks. The cleaned scale was wet mounted on a slide with a coverslip, and imaged with a Nikon confocal microscope. Scales were centered on the microscope under 10x (for the largest scales, often species of *Percina*) or 20× magnification with transmitted light. We auto-scaled the images using NIS Elements b925 (version 4.13.04) before scanning. If scales were not in sharp focus within NIS Elements, we manually focused them before the image was captured. Using similar methods to Ibáñez (2015), we placed seven geometric morphometric landmarks on each image using tpsDig2 version 2.26 (Rohlf 2005; Fig. 1). These included a landmark at each of the dorsal and ventral boundaries of the ctenial margin and scale body (Fig. 1, landmarks 1 and 5, respectively), one at the base of the ctenus at the apex of the curve of the ctenial margin (landmark 2), one at the original base of this same ctenus (at the apex of the curve of the ctenial margin; landmark 3), one at the center of the scale focus (landmark 4), and one each at the anterior end of the scale at the ventral- and dorsal-most radii (landmarks 6 and 7, respectively). We used a Procrustes superimposition to remove the effects of scale, size, and rotation (Zelditch et al. 2012) in program R version 4.1.1 (R Development Core Team 2021) with package *geomorph* version 4.0.0 (Adams et al. 2021). We analyzed scale shape disparity within and among all darter species using the *morphol.disparity* function in *geomorph*, comparing disparity based on the group mean for each species to disparity based on the overall mean for all species. Except for *Ammocryta beani* (a very morphologically distinct species in body and scale shape, with the least amount of scales among all darters), we found that morphological disparity within species was less than that of overall disparity for all species (Appendix 2). Given that the disparity analysis results provide evidence for low intraspecific variation relative to interspecific variation in scale shape, and that our focus was on patterns of scale shape variation among darter species, intraspecific variation was not explored further. Additionally, we could not test for effects of sex on scale shape due to missing sex data or unequal numbers of males and females available, so we averaged Procrustes coordinates within each species.

Ordinations and comparative analyses

To examine overall scale shape variation, and scale shape patterns related to phylogeny, we generated a covariance matrix from the averaged Procrustes coordinate shape data for all species to run a Principal Component Analysis (PCA) with default settings in *vegan* version 2.5-7 (Oksanen et al. 2020). Using Mesquite version 3.51 (Maddison and Maddison 2018) we pruned the darter phylogeny from Near et al. (2011) to the full 92 species selected in our study and pruned again to include only those in our study that were benthic in WCP (72 species total). Component scores for each species were plotted in PCA phylo-morphospace to visualize variation in scale shape among and within genera and terminal clades within genera of darters (sensu Near et al. 2011). We employed a “Kmult” test (R

package *geomorph*; Adams et al. 2014) to quantify and evaluate the significance of phylogenetic signal in darter scale shapes against the null hypothesis of a Brownian Motion model of evolution ($K = 1$). To determine whether scale shape covaries with ecological variables after accounting for shared ancestry, we conducted a Phylogenetic Generalized Least Squares (PGLS) with RRPP randomization and SS type (III) using the function `procD.pgls` in *geomorph* (Adams and Collyer 2015, 2018; Zelditch et al. 2012, 2017). In this analysis our averaged scale shape for each of the 92 darter species were regressed against the effects of all selected ecological variables with a full PGLS model of $y = \text{size} + \text{ecosystem type} + \text{microhabitat} + \text{spawning mode} + \text{WCP} + \text{substrate}$. Any non-significant variables were removed to find the most parsimonious PGLS model. A null PGLS model was compared to the parsimonious PGLS model in an ANOVA table (Appendix 3) to evaluate significance. Due to the likely influence of both phylogeny and ecology on darter scale shape, we conducted a Phylogenetic Principal Component Analysis (Phy-PCA). The Phy-PCA was used to assess whether the major axis of scale shape variation was related to any non-phylogenetic effects, specifically our selected ecological variables (Collyer and Adams 2020). Species scale shape scores were plotted in phylomorphospace for the Phy-PCA and coded by each ecological variable type. We carried out all analyses on both the full 92 darter species and reduced 72 benthic darter species datasets. Our model for the PGLS of the benthic darters, was identical to that of the 92 species set, but excluded WCP as a variable, since all species were benthic. Given the large taxonomic scope and ecological breadth of our data and particularly, the coarseness of our ecological variables, our statistical power to detect relationships between scale shape and ecology were reduced. Therefore, we chose an α -level of 0.1 to determine if p-values were significant in all applicable comparative analyses.

Results

Scale shape variation, ecological, and phylogenetic signal among all darters

We generated scale shape data from 92 species that represent all six darter genera and 37 terminal clades within these genera sensu Near et al. (2011), and all variables (ranging from 2 to 4) of each ecological variable examined (Table 1). In the Principal Component Analysis (PCA) of all darters the first two Principal Component (PC) axes of scale shape variation among all species explained 82.4% of the variation (Fig. 1). Scale shape change along PC axes is summarized by transformation grids, where the antero-posterior length of the scale increases and the dorso-ventral width decreases (scales become longer and thinner; Fig. 1) with increasingly positive values along PC1. Scales become shorter and wider with negative values along the PC1 axis. Darters with positive scale shape scores along PC2 had scales with narrowed anterior scale insertions and an enlarged (longer, wider) ctenial margin that was more recessed anteriorly into the body of the scale, while species with negative scores along PC2 had shorter, narrower ctenial margins that protruded more from the scale body posteriorly (Fig. 1). Considerable variation was observed in shape both within and among darter genera and their terminal clades (Fig. 1; see Table 1 for terminal clade designations). Several taxa had particularly distinct scale shapes, including the sub-benthic *Ammocrypta beani* (01) and *A. pellucida* (02), with recessed and enlarged ctenial margins, and narrow anterior scale insertions compared to other species. Though closely related to *A. beani* and *A. pellucida*, *A. vivax* (03) and *Crystallaria asprella* (04) had scale

shape scores similar to species from other genera (Fig. 1), but their scales still followed the general shape pattern of being narrowed anteriorly with an enlarged ctenial margin. *Nothonotus tippecanoe* (69), *E. vitreum* (59), *E. microperca* (38), and *E. proeliare* (46), had short, wide scales recovered more negatively along the PC1 axis (Fig. 1). In contrast, all species of *Ammocrypta* (01, 02, 03), *E. sagitta* (50), and all members of clade *Richiella* (13, 31, 51) scored more positively along PC2 than most species. Several species of genus *Percina* (70, 71, 76, 77, 79, 82, 85, 87, 88, 90) and one species of *Etheostoma* (*E. parvipinne* (43)) had long, thin scales that scored positively along PC1 (Fig. 1). However, there was a large cluster of primarily benthic darters, comprised mostly of *Etheostoma*, *Nothonotus*, and a small subset of *Percina* species around the origin of the graph (e.g., those bounded by -0.1 to 0.1 on PC1 and by approximately -0.05 to 0.05 on PC2) where species had square scales that diverged little from the overall average scale shape of all darters examined (Fig. 1).

The PCA showed examples of close relatives with similar scale shapes (e.g., the clade *Richiella* from within genus *Etheostoma* (13, 31, 51) clustered in the negative region of PC1 and the positive region of PC2; species of clade *Microperca* from within genus *Etheostoma* (38, 46) clustered together, having negative PC1 and PC2 loading scores; and many species from the clade *Hadropterus* in the genus *Percina* (77, 82, 87, 89) had similar scale shapes with positive loading on PC1; Fig. 1). Although some related species of darters had similar scale shapes, overall we found that species were less similar than expected under a pure Brownian Motion model of evolution ($K=0.6801$, $p=0.001$).

Several closely related species pairs such as *P. palmaris* (83) and *P. lenticula* (77) of clade *Hadropterus*; *P. shumardi* (88) and *P. vigil* (92) of clade *Imostoma*; *E. blennioides* (11) and *E. swannannoa* (56) of clade *Neoetheostoma*; and *N. microlepidus* (67) and *N. tippecanoe* (69) had divergent scale shapes (Fig. 1). In many cases, these closely related pairs of species were also different in one or more ecological variables. Additionally, some distant relatives including *Crystallaria asprella* (04) and *E. swannannoa* (56); *N. tippecanoe* (69) and *E. vitreum* (59); and *E. baileyi* (07) of an unnamed clade within the *Simoperca* clade), *E. fusiforme* (25) of clade of clade *Hololepis*, and *E. planasaxatile* (44) of clade *Ulocentra*) had similar scale shapes and ecologies (Fig. 1). Divergence between close relatives and potential convergence between distant relatives in scale shape suggested factors other than phylogeny alone contributed to scale shape variation in darters.

In the full PGLS model for all species of darters, both size ($r^2=0.12$, $p=0.001$) and WCP ($r^2=0.09$, $p=0.001$) were significantly related to scale shape variation after accounting for phylogeny (Appendix 3a). In the parsimonious PGLS model (including only WCP and body size), size ($r^2=0.13$, $p=0.001$) and WCP ($r^2=0.12$, $p=0.001$) were significant (Appendix 3b). When the parsimonious PGLS model was compared with a null PGLS model, the parsimonious model was significantly different from the null (Appendix 3c), indicating that WCP and body size are responsible for some of the divergence and possible convergence in scale shape among our darter specimens.

Results of the Phy-PCA were similar to those in the PCA plotted in phylomorphospace (Fig. 1) with most of the variation in scale shape distributed along PC1 (67.6%), and 11.6% of variation explained by PC2 (Fig. 2a, b). Scale shape changes along the PC1 and PC2 axes, summarized by the transformation grids (Fig. 2a, b), also were similar to the shape changes for the all species PCA (Fig. 1). We coded the Phy-PCA output by ecological variables for the two variables that were significant in the PGLS analysis of all darters (WCP and body size; Fig. 2a, b). Scale shape variation was observed among species in each water column position (WCP; Fig. 2a) and body sizes (Fig. 2b) with considerable overlap among

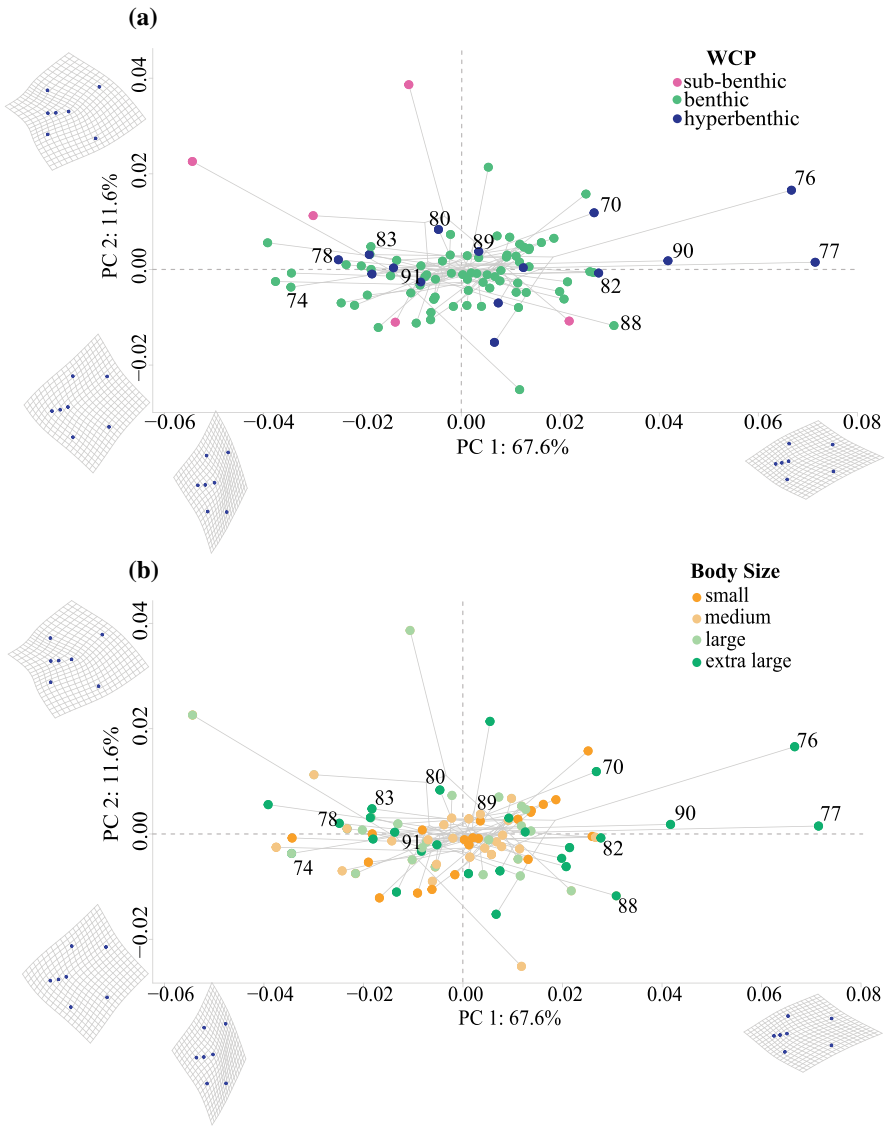


Fig. 2 Phylogenetic PCAs (Phy-PCA) of scale shape variation for all 92 species of Etheostomatinae darters with the average scale shape for each species coded by ecological variables of water column position and body size. Numbers identify species codes and correspond to those in Table 1. Transformation grids associated with graph axes depict scale shape change from the overall average scale shape of all darters along each axis. Grey lines connecting points represent the phylogenetic relationships among species based on Near et al. (2011). **a** Phy-PCA of all species examined with averaged scale shape scores of species coded by WCP. **b** Phy-PCA of all species examined with averaged scale shape scores of species coded by body size groups. Other ecological variables examined showed considerable overlap in morphospace for scale shape variation and were not significant in the PGLS and are not shown

all groups for both variables. However, three of the five sub-benthic darters clustered in the positive region of PC2, having scales with narrow anterior insertions and enlarged ctenial margins in the Phy-PCA (Fig. 2a). Additionally, a long, thin scale shape pattern was associated with multiple species that were both hyperbenthic and extra-large (average SL between 56 and 121 mm) in size. All extra-large, hyperbenthic darters with long, thin scales were species from genus *Percina* (e.g. 70, 76, 77, 82, 90 Fig. 2a, b). Species coded by spawning mode, environment type, substrate, or microhabitat had considerable overlap in variation along both axes and were not significant in the PGLS, so Phy-PCAs coded by these variables are not shown.

Scale shape variation, ecological and phylogenetic signal among benthic darters

We generated scale shape data from 72 benthic species that represent three darter genera and 30 terminal clades within these genera, sensu Near et al. (2011), and all variables (ranging from 2 to 4) of each ecological variable examined (except WCP). In the PCA of benthic darters, the first two PC axes explained 79.3% of the scale shape variation (Fig. 3a). Scale shape change along PC1 and PC2 is summarized by transformation grids (Fig. 3a) and closely follows trends seen in the PCA of all species (Fig. 1). There was considerable variation within each benthic genus, but *Nothonotus* species were primarily associated with the negative region of PC1 and positive region of PC2 (Fig. 3a). Benthic *Percina* were primarily associated with the positive region of PC2, but broadly distributed across PC1 (Fig. 3a). *Etheostoma* had scale shape variation spanning both the positive and negative regions of both axes (Fig. 3a). As in the all species PCA, we found examples of close relatives with similar scale shapes (e.g., *E. bison* (10), *E. lawrencei* (33), and *E. spectabile* (53) from the clade *Ceasia*; and *E. microperca* (38) and *E. proeliare* (46) from the clade *Microperca*; Fig. 3a). Although some related species of benthic darters had similar scale shapes, overall we found that species were less similar than expected under a pure Brownian Motion model of evolution ($K=0.671$, $p=0.001$).

We found several examples of closely related species with divergent scale shapes (e.g., *N. acuticeps* (63) and *N. tippecanoe* (69); *P. shumardi* (88) and *P. vigil* (92) from clade *Imostoma*; and *E. gutselli* (27) and *E. lynceum* (36) from clade *Neoetheostoma*; Fig. 3a). In many cases, these closely related species pairs were also different in one or more ecological variables (Table 1). There were also examples of more distant relatives, including examples from different genera, with similar scale shapes (e.g., *E. lynceum* (36), *N. bellus* (64), *P. copelandi* (72 of clade *Cottogaster*), and *P. roanoka* (86 of clade *Atlantis*) had similar, slightly shortened and widened scales; *E. sitikuense* (51) and *N. acuticeps* (63) had square shaped scales with enlarged ctenial margins; and *E. parvipinne* (43) and *P. shumardi* (88) both had similar long, thin, scales; Fig. 3a) and ecologies (Table 1). As in the all species analyses, divergence between close relatives and potential convergence between distant relatives suggested factors other than phylogeny alone contributed to scale shape variation in darters.

In the full PGLS model for benthic species of darters only, size ($r^2=0.08$, $p=0.002$) was significantly related to scale shape variation after accounting for phylogeny (Appendix 3d). In the parsimonious PGLS model (including only body size), size ($r^2=0.07$, $p=0.007$) was significant (Appendix 3e). When the parsimonious PGLS model was compared with a null PGLS model, the parsimonious model was significantly different from the null (Appendix 3f), indicating that body size was responsible for some of the divergence and possible convergence in scale shape among our darter specimens.

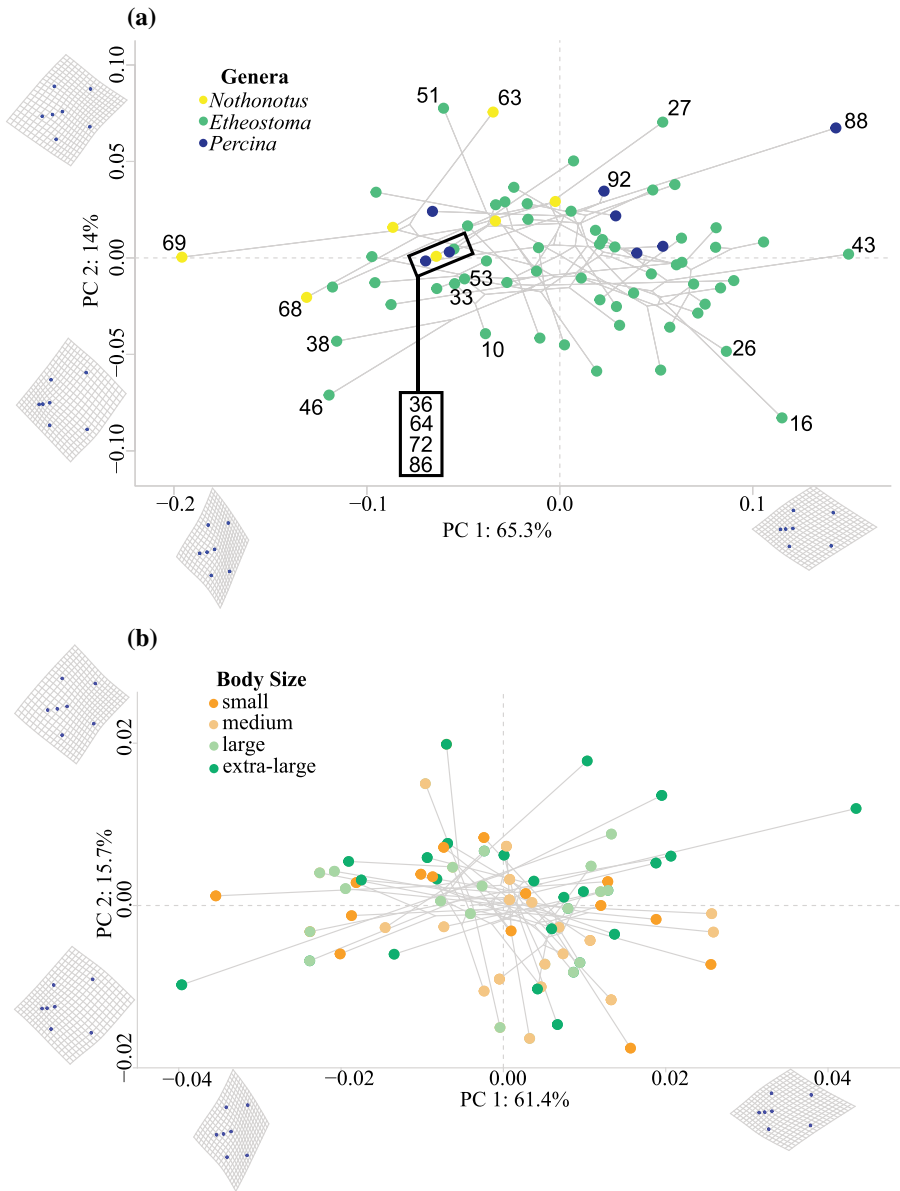


Fig. 3 Principal component analysis (PCA) and Phylogenetic PCA (Phy-PCA) of scale shape variation for the 72 benthic species of Etheostominae darters with the average scale shape for each species, represented by dots. Transformation grids associated with graph axes depict scale shape changes from the overall average scale shape of all darters along each axis. Grey lines connecting points represent the phylogenetic relationships among species based on Near et al. (2011). **a** PCA in phylomorphospace of benthic species examined with averaged scale shape scores for each species coded by genus. **b** Phy-PCA of benthic species examined with averaged scale shape scores for each species coded by body size. Other ecological variables examined were not significant in the PGLS and showed considerable overlap in morphospace for scale shape variation and are not shown

Results of the benthic Phy-PCA were similar to those in the PCA plotted in phylomorphospace (Fig. 3a) with most of the variation in scale shape distributed along PC1 (61.4%), and only 15.7% of variation explained by PC2 (Fig. 3b). Scale shape changes along the PC1 and PC2 axes, summarized by the transformation grids (Fig. 3b), also were similar to the transformation grids in the all species and benthic PCAs (Figs. 1, 3a). We coded the Phy-PCA output by the only ecological variable that was significant in the PGLS analysis of benthic darters (body size; Fig. 3b). Scale shape variation was observed among species with different body sizes (Fig. 3b), but there was considerable overlap among all size groups. Despite a significant association between scale shape and size in the PGLS, no distinct clustering among sizes was found in the Phy-PCA (Fig. 3b). Species coded by spawning mode, environment type, substrate, or microhabitat had considerable overlap in variation along both axes and were not significant in the PGLS, so Phy-PCAs coded by these variables are not shown.

Discussion

We expected evolutionary history to contribute to patterns of variation in scale shape, given that phylogeny explains some variation in darter body shapes among genera and subgenera (Guill et al. 2003) and is a strong predictor of phenotypic traits in many other aquatic and terrestrial taxa including Monogenean haptor anchors (fish parasites; Rodríguez-González et al. 2017) and Scarab beetle wings (Ospina-Garcés et al. 2018). As predicted, we observed clades with members that share similar scale morphologies (Fig. 1) and detected a significant, though weak, phylogenetic influence on scale shape. Thus, scale shape, similar to body shape in darters and phenotypic traits of many other taxa, is influenced by phylogeny. The weakness of the signal might be partially due to within-species variation which we did not account for explicitly. However, divergent scale shapes between close relatives (i.e., *N. microlepidus* and *N. tippecanoe*) and similar scale shapes shared by distant relatives (i.e., *E. vitreum* and species of genus *Ammocrypta*) suggest environmental factors or behavior may also contribute to observed patterns of scale shape variation in darters.

One such environmental factor could be water column position (WCP), which was significantly related to scale shape, after accounting for phylogenetic signal (in the PGLS). Hyperbenthic species had elongated scales relative to benthic and sub-benthic species, with the latter having scales with narrowed anterior bases and enlarged ctenial margins. The influence of WCP on morphological trait variation has been previously observed among darters and other aquatic taxa. For example, both closely and distantly related darters that bury in sand (sub-benthic) share slender bodies, small fins, a narrow inter-orbital width, similar scale microstructure morphology, and a loss or reduction in scale number and size (Kuehne and Barbour 1983; Page and Swofford 1984; Coburn and Gaglione 1992; Figs. 2a, 4). These sub-benthic darters (especially species of the genus *Ammocrypta* and *E. vitreum*) spend much time hidden below the substrate, where they experience reduced predation and viscous drag (Page and Swofford 1984). Increased selection on traits that improve burying efficiency (such as those that reduce skin friction) and (or) a release from selective pressures of predation and viscous drag has led to convergence in morphological traits among burying species of darters (Kuehne and Barbour 1983; Page and Swofford 1984; Spinner et al. 2016). Similarly, a benthic WCP has been associated with brighter coloration, less fusiform bodies, and larger pectoral fins (Page and Swofford 1984). The increase in body

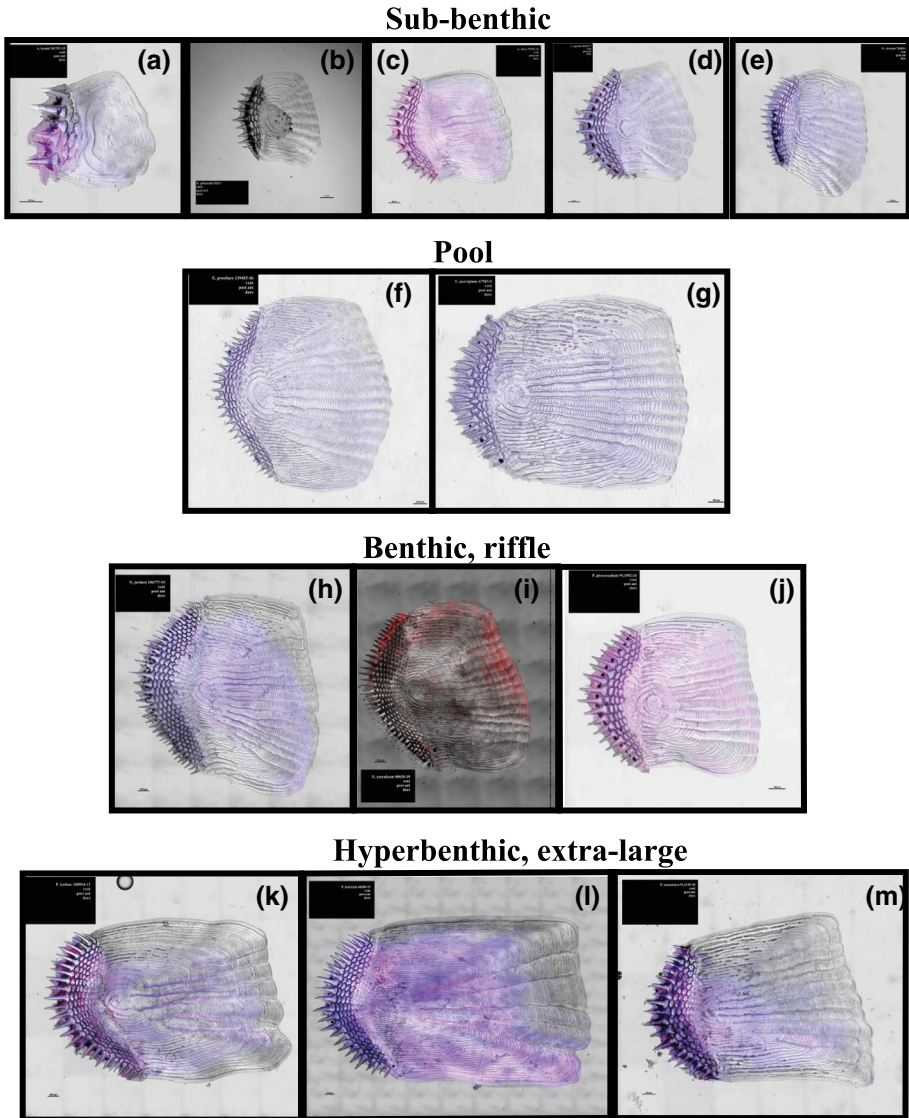


Fig. 4 Examples of darter scale shape variation observed for the different ecological variables examined including those that were classified as Sub-benthic in water column position (a–e), restricted to pool microhabitats (f, g) or benthic, riffle habitats (h–j), and extra-large, hyperbenthic species (k–m). Species (and species codes from Table 1) represented by photos are: **a** *Ammocrypta beani* (01), **b** *A. pellucida* (02), **c** *A. vivax* (03), **d** *Crystallaria asprella* (04), **e** *Etheostoma vitreum* (59), **f** *E. proeliare* (46), **g** *E. parvipinne* (43), **h** *Nothonotus jordani* (66), **i** *E. caeruleum* (14), **j** *Percina phoxocephala* (84), **k** *P. kathae* (76), **l** *P. lenticula* (77), and **m** *P. aurantiaca* (70)

coloration may result from relaxed pressures of predation, and the loss of fusiformity may result from relaxed pressures relating to flow velocity, due to boundary layer usage, while large pectoral fins may be important for increasing grip on the bottom of a stream (Page and Swofford 1984; Bossu and Near 2015). Hyperbenthic darters are the most exposed to

flow and predators, and share features such as smaller pectoral fins, more fusiform body shapes, and cryptic coloration which may result from selective pressures of flow and predation (Page and Swofford 1984; Bossu and Near 2015). In marine systems, fishes including snappers (Lutjanidae), show convergence of phenotypes by water column position: benthic species have deep bodies and truncate caudal fins and pelagic species have slender-bodies with furcate caudal fins (Rincon-Sandoval et al. 2020). Also, pelagic damselfishes in laminar flow habitats have scales with smoother surfaces than benthic damselfishes in turbulent flows, suggesting modifications to scale rugosity occur based on a combination of water column position and flow type occupied (Wainwright 2019). In summary, various selective pressures associated with water column position use, such as predator avoidance and drag reduction, can influence body shape, coloration, and fin and scale morphology of fishes (Page and Swofford 1984; Bossu and Near 2015; Wainwright 2019; Rincon-Sandoval et al. 2020). Although not explicitly tested, it is possible that the unique scales of sub-benthic darters (enlarged cteneal margin and narrowed anterior insertion; Figs. 1, 2a, 4) also aid in burying efficiency and (or) predator avoidance similar to other shared traits found among sub-benthic fishes. Similarly, the long, thin scales of hyperbenthic darters (Figs. 1, 2a, 4) may aid in drag reduction, increased steady-state swimming efficiency, and (or) predator avoidance, similar to other shared traits found among hyperbenthic fishes. However, additional studies are needed to explicitly test these hypotheses.

Body size was associated with scale shape variation among all darters and benthic darters after accounting for phylogeny (PGLS). The extra-large darters (those with SL between 56 and 121 mm) primarily drove this result in the all-species analysis. Darters with the longest and thinnest scale shape along PC1 were both hyperbenthic and extra-large in size (Fig. 2a, b). Body size is positively correlated with drag, so larger species experience increased drag forces relative to smaller species (Webb 1988). Additionally, larger fishes may have less ability to fully shelter in the boundary layer (Carlson and Lauder 2011), and are more exposed to flow and drag forces relative to smaller darters. We might expect from previously noted patterns in body and fin shapes of fishes that species with long, thin scale shapes will also inhabit high-flow environments (Casatti and Castro 2006; Langerhans 2008; Leal et al. 2011; Pagotto et al. 2011; Bower et al. 2021). However, the extra-large, hyperbenthic species (mostly genus *Percina*; Figs. 1, 2a, b, 4) with the longest and thinnest scale shapes primarily are generalists, or specifically occupy slow-flowing pools (Table 1). Fishes living in slower flow regimes, such as pools, typically have deeper body shapes that maximize unsteady-state (highly maneuverable) swimming (Brinsmead and Fox 2002; Langerhans 2008; Foster et al. 2015; Wainwright 2019). But, extra-large, hyperbenthic darters that occupy lower-flow habitats are characterized by elongated, stream-lined bodies (Page 1983). Their body shape is likely associated with their behavior of actively swimming above the substrate, out of the boundary layer, and in the water column where they experience regular unidirectional, or laminar flow (Krabbenhof et al. 2009; Meyers and Belk 2014). Adaptations that reduce drag or increase steady-state swimming efficiency, such as a more fusiform body shape, are predictable traits for fishes that not only experience high flows but also laminar flows in their environment (Langerhans 2008; Krabbenhof et al. 2009; Meyers and Belk 2014). The commonality of long and thin-bodied darters that are habitat generalists, further suggest that flow type (laminar or turbulent) and not just flow velocity contributes to trait evolution in aquatic organisms (Page 1983; Wainwright 2019). We found that the scale morphology of the largest, hyperbenthic darter species mirrors body morphology (since these fishes have long, thin scales (Fig. 4) and long, stream-lined bodies). Therefore, scale shape may also reflect adaptations to laminar flows experienced while roving in the water column.

Some extra-large, hyperbenthic darter species such as *P. macrocephala* (P78) and *P. maculata* (P80), did not have long, thin scales (Fig. 2a, b). Based on their body size and water column position, we expected both of these species to have the long, thin scale shape of other large, hyperbenthic darters. However, *P. macrocephala* and *P. maculata* are often associated with shelter use (water willows, large rocks, woody debris), and a lower level of roving activity (Etnier and Starnes 1993). Shelter use may contribute to a reduction in laminar drag forces experienced by *P. macrocephala* and *P. maculata*, resulting in a deviation from the long-thin scale shape of other extra-large, hyperbenthic darters. Additionally, *Percina shumardi* (P88), a primarily benthic, but extra-large darter, shared the long, thin scale shape of the other larger bodied, hyperbenthic darters (Figs. 1, 2a, b). *Percina shumardi* are usually found in large, high-flow river environments with fine substrates that likely provide reduced boundary layer shelter from flow (Hoerner 1965; Carlson and Lauder 2011; Bower 2021). Thus, *P. shumardi* may experience similar flow-related selection pressures of the other large bodied, hyperbenthic darters. However, most darters likely occupy a spectrum between the benthic and hyperbenthic zones of streams and therefore, the scale shape of *P. shumardi* may instead (or also) indicate that this species spends more time above the benthos than previously thought. Importantly, we observed both extra-large, benthic species (e.g., *P. palmaris* (P83)), and smaller, hyperbenthic species (e.g., *P. stictogaster* (P91), *P. smithvanizi* (P89)), that did not have long, thin scales characteristic of fishes that are both hyperbenthic and extra-large in size (Fig. 2a, b). This indicates that for some darters, scale shape likely reflects selection imposed through a combination of both WCP and body size. Myers and Belk (2014) also found that interactions between WCP and body size drive morphological variation among body shapes of Mountain sucker (*Catostomus platyrhynchus*) at intraspecific levels, confirming that the interaction of body size and WCP can contribute to predictable patterns of variation in morphological traits over short periods of time.

After accounting for phylogenetic signal in the benthic-only dataset (PGLS), body size was the only ecological variable significantly associated with scale shape. However, we observed considerable overlap among benthic darter body sizes in the Phy-PCA (Fig. 3b) and many of our other taxonomic and ecological categories (Fig. 4). Failure to detect a relationship between scale shape and ecological factors may be an artifact of boundary layer usage by benthic species (Carlson and Lauder 2011). The lack of clear associations between darter scale morphology and ecology could also be due to placing species in coarsely-defined groups, especially considering that measurable body shape variation in some darters has been documented across highly nuanced environmental gradients and narrow geographic areas (Hopper et al. 2017). Future studies that specifically test the relationship between different scale shapes as well as other features like scale rugosity (Wainwright 2019) and color (Bossu and Near 2015), and size or shape of the ctenial margin, with different environmental conditions will help elucidate the adaptive significance of fish scales.

Overall, our study suggests that scale shape in darters is influenced by phylogeny, as well as habitat use and interactions between habitat (particularly water column position) and body size. The observed link between scale shape and WCP and size implies that the scales of Teleost fishes, like those of placoid scales in sharks, may play a vital role in drag reduction, facilitating movements, through different flow types in general. In benthic species, observations of considerable shape variation among species and lack of support for an influence of flow type experienced on scale shape suggests use of the boundary layer may reduce selective pressures of flow on scale shape.

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Data availability Upon acceptance to journal, any data which supports the findings of this study will be deposited in Dryad.

Code availability Code will be available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication All authors consent to this research being published.

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