

Evolutionary patterns of scale morphology in damselfishes (Pomacentridae)

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Fish scales are bony plates embedded in the skin that vary extensively in shape across taxa. Despite a plethora of hypotheses regarding form–function relationships in scales, we know little about the ecological selective factors that shape their diversity. Here we examine evolutionary patterns of scale morphology using novel three-dimensional topography from the surfaces of 59 species of damselfishes, a prominent radiation of coral reef fishes. We find evidence that scale morphology changes with different flow environments, such that species that spend more time in open-water habitats have smoother scales. We also show that other aspects of ecology lead to highly derived scales. For example, anemonefishes show an evolutionary transition to smaller scales and smaller ctenii (scale spines). Moreover, changes in body shape, which may reflect ecological differentiation, are related to scale shape but not surface properties. We also demonstrate weak evolutionary integration among multiple aspects of scale morphology; however, scale size and shape are related, and scale morphology is correlated between different body regions. Finally, we also identify a relationship between aspects of lateral line pore morphology, such that the number of lateral line pores per scale and the size of those pores are inversely related. Overall, our study provides insights into the multidimensionality of scale evolution and improves our understanding of some of the factors that can give rise to the diversity of scales seen across fishes.

ADDITIONAL KEYWORDS: biological surfaces – functional morphology – ichthyology – profilometry.

INTRODUCTION

Fish scales are bony overlapping plates embedded in the epidermis, and they can differ in size and shape, as well as in the presence and orientation of spiny projections or ridges (Roberts, 1993). Suites of these attributes tend to vary together in major groups of fishes, creating broad categories of scales, such as smooth-edged cycloid scales or spiny-edged ctenoid scales, though substantial variation exists within these categories (Johnson, 1984; Roberts, 1993). Indeed, scale morphology has been shown to vary considerably at multiple taxonomic levels: among species (Agassiz, 1833; Cockerell, 1911; Kobayasi, 1955; Roberts, 1993; Daniels, 1996; Lippitsch, 1998), among populations within species (Richards & Esteves, 1997; Poulet *et al.*, 2005), between juveniles and adults (Pothoff &

Kelley, 1982; Tyler *et al.*, 1989; Frédérick *et al.*, 2010), and even across different body regions on the same individual (Dapar *et al.*, 2012; Wainwright & Lauder, 2016; Wainwright *et al.*, 2018). In fact, many species possess spiny ctenoid and smooth-edged cycloid scales on different regions of their body (Lippitsch, 1998; Ibañez *et al.*, 2009; Wainwright & Lauder, 2016; Viertler *et al.*, 2021). Overall, scale diversity remains an important ichthyological tool, with differences in scale placement, morphology and meristics often used for distinguishing closely related species, and larger differences in scale morphology, type and presence distinguishing family-level clades.

Despite widespread recognition of scale form as a prominent aspect of fish morphological diversity, we still lack knowledge of three-dimensional (3D) quantitative scale morphology and have limited insights into how scale diversity arises through evolution. Although there is a strong history of research on fish scale morphology, few studies have described

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scale morphology using the kinds of quantitative measurements that would facilitate comparisons among species or across studies (although see [Viertler *et al.*, 2021](#)). In fact, until the recent adoption of profilometry methods that provide 3D data for fish surfaces ([Sudo *et al.*, 2002](#); [Wainwright *et al.*, 2017](#)), we have not been able to fully explore variability in the surface texture of fish scales. Moreover, this paucity of quantitative interspecific morphological data has limited the use of modern phylogenetic comparative methods to explore the factors that shape scale diversity. In this study, we quantify the topography of scaled surfaces in an ecologically diverse clade as a way to describe patterns of scale evolution and test hypotheses about the selective pressures that shape scale diversification.

One step in understanding scale diversity is to link structural variation with functional consequences, and a variety of functional hypotheses for scales have been proposed. Scales and fish surfaces are multifunctional structures with purported roles in protection from predators ([Browning *et al.*, 2013](#); [Vernerey & Barthelat, 2014](#)) and calcium storage ([Parenti, 1986](#)), with other studies suggesting that scales could influence water flow around swimming fishes ([Aleyev, 1977](#); [Burdak, 1986](#); [Wainwright & Lauder, 2016, 2018](#); [Wu *et al.*, 2018](#); [Muthuramalingam *et al.*, 2019](#)). Fish scales also play an important sensory role by housing the lateral line trunk canal in modified scales ([Webb & Ramsay, 2017](#)). The lateral line canal allows fishes to sense spatial pressure differences (from flow acceleration) that are transmitted through external pores that lead into the enclosed canal ([van Netten & McHenry, 2013](#); see also Methods). Scales can also have a number of different microstructures, and previous work has hypothesized that spines, ridges or scales themselves project into the flow to either decrease drag or increase thrust in turbulent flows ([Burdak, 1986](#); [Ibañez *et al.*, 2009](#); [Lauder *et al.*, 2016](#); [Wainwright & Lauder, 2016](#); [Wu *et al.*, 2018](#)). In addition, some recent studies using modelling and experiments have attempted to confirm hypotheses of drag reduction for scaled surfaces ([Wu *et al.*, 2018](#); [Muthuramalingam *et al.*, 2019](#)). These studies suggest that along with other specific protective, physiological and sensory functions, scale morphology may have important consequences for flow around fish as they move through the environment. In summary, if scale morphology affects function and performance, then shifts along different axes of ecology should lead to changes in scale morphology.

In addition to the selective factors that affect scale evolution, there may also be evolutionary integration that affects the combinations of scale forms that we see among species. Evolutionary integration is reflected in the pattern of correlations in evolutionary change of particular morphological variables and is a consequence

of functional integration, shared developmental pathways, genetic correlation or correlated selection ([Cheverud, 1996](#); [Wagner *et al.*, 2007](#)). Not much is definitively known about these different sources of integration for scales. Work on scale development has revealed the basis for major qualitative differences in scale morphology [e.g. the loss of scales in catfishes or the origin of spines and plates in tetraodontiformes ([Liu *et al.*, 2016](#); [Aman *et al.*, 2018](#); [Shono *et al.*, 2019](#))], but this work has not yet addressed quantitative scale variation we see at finer taxonomic levels, perhaps due to a lack of information on the nature of interspecific scale variability. Similarly, we lack clear expectations about how multiple dimensions of scale morphology should covary during evolution, due to genetic or functional constraints. Although the current literature does not specify particular paths for evolutionary integration in scales, our study helps document patterns of morphological correlations as a starting point for determining which sources of integration are important for scales.

For this study, we examine scale evolution in the teleost fish family Pomacentridae (damselfishes), which is a diverse family of over 380 species ([Froese & Pauly, 2018](#)) that mostly occur on coral reefs and exhibit repeated transitions between open-water and structure-associated feeding ([Frédérich *et al.*, 2013](#)). Damselfishes are well studied; we have a good understanding of their phylogeny from the use of molecular data ([Tang, 2001](#); [Cooper *et al.*, 2009](#); [Frédérich *et al.*, 2013](#); [Tang *et al.*, 2021](#)), and a range of ecological, morphological and functional diversity in damselfishes has been described ([Emery, 1973](#); [Atkinson & Grigg, 1995](#); [Ormond *et al.*, 1996](#); [Gluckmann & Vandewalle, 1998](#); [Frédérich *et al.*, 2008](#); [Barneche *et al.*, 2009](#); [Cooper & Westneat, 2009](#); [Aguilar-Medrano *et al.*, 2013](#); [Aguilar-Medrano *et al.*, 2016](#)). For example, damselfishes are known to be diverse in their feeding ecology: some species mostly capture zooplankton, while others feed on benthic organisms on the reef ([Allen, 1975](#); [Frédérich *et al.*, 2009](#); [Gajdzik *et al.*, 2016](#)). In addition, damselfishes show a diversity of body shapes, which are commonly related to differences in ecology, and they have evolved some novel ecologies, such as colonizing anemones and algal farming. The multiple axes of ecological diversity and the strong history of studying damselfishes make them an ideal group to explore the evolution of scale diversity.

We examine evolutionary patterns of scale morphology using both directed analyses to test for connections between morphology, ecology and function, and a general exploration of the evolutionary dynamics of scale form. We present 3D topographic data describing surface features of scales from a diverse sample of damselfish species that represents the major phylogenetic lineages

within the group and multiple ecological transitions. We analyse these data using modern phylogenetic comparative analyses that account for uncertainty in species trait values to better understand how scale diversity is generated and maintained. More specifically, we develop analyses to address two central aims:

1. What ecological factors affect the evolution of scale morphology?
2. What are the patterns of evolutionary integration among scale characteristics?

With this study, we move beyond general classifications of scale variation and take advantage of novel 3D imaging methods and modern comparative analyses to identify previously unrecognized dimensions of scale diversity and their evolutionary origins. For example, we use data-driven evolutionary model-fitting analyses to identify transitions in adaptive optima for scale morphology (Uyeda & Harmon 2014; Khabbazzian *et al.*, 2016) and ask if those transitions overlap with ecological shifts, thus more broadly evaluating potential ecomorphological relationships beyond targeted functional hypotheses. We also examine the basis of scale diversity through the analysis of evolutionary correlations between aspects of scale morphology. This analysis can identify suites of attributes that evolve together and could reveal developmental or functional constraints and integration on particular axes of scale form. By studying how scale morphology changes with canonical axes of diversity such as ecology and body shape, and by quantifying scale diversity using novel 3D imaging methods, we can start to solve the age-old question in fish biology of why fish scales are so diverse.

MATERIAL AND METHODS

SPECIMEN SAMPLING

We sampled three to five adult individuals from each of 59 species of damselfishes from the Museum of Comparative Zoology's (MCZ) Ichthyology collection at Harvard University (Supporting Information, Table S1). All specimens were previously preserved in formalin and stored in 70% ethanol. We sampled from all damselfish genera with over ten species, and we sampled species that are probably descended from multiple independent evolutionary transitions in feeding ecology, as inferred from the most recent and complete molecular phylogeny of the group (Frédérich *et al.*, 2013).

IMAGING OF SCALES

Surface topography and 3D information of scales is crucial to making informative hypotheses about their function, yet our knowledge of the 3D structure of fish

skin is currently limited (Sudo *et al.*, 2002; Sagong *et al.*, 2008; Liyan *et al.*, 2017; Wainwright *et al.*, 2017). To capture surface topography, we used a gel-based profilometer manufactured by GelSight Inc. (Waltham, MA, USA). This technique can accurately reconstruct surface features of fish scales and other biological surfaces (Wainwright & Lauder, 2016; Wainwright *et al.*, 2017, 2019; Baeckens *et al.*, 2019). Briefly, gel-based profilometry works by pressing a soft gel with a painted bottom surface into the surface of interest. Six images are taken under lighting from different angles (Johnson & Adelson, 2009; Johnson *et al.*, 2011), and GelSight software uses these six images to reconstruct surface topography (Fig. 1).

For each specimen, we imaged the body surface and scales embedded in it on the left side of the body at two locations: a midbody region above the dorsal margin of the pectoral fin and including a portion of the lateral line trunk canal, and a posterior region directly anterior of the narrowed caudal peduncle (Fig. 1A). We captured the surface topography of these regions, allowing us to measure characteristics in one, two and three dimensions. To remove topography due to curvature of the body of the fish, we fitted a polynomial (6–12 degrees) to the surface using the 'remove form' function of the MountainsMap software (v.7.2.7344, Digital Surf, Besançon, France). This polynomial-fitted surface was removed from the measured surface to obtain only topography of surface features, such as scales, and not topography due to the curved surface of the fish. We then used MountainsMap to take and record linear measurements, as well as surface metrology measurements such as root-mean-square roughness, skew and kurtosis (see next section).

To prepare surfaces for gel-based profilometry, midbody and posterior regions were lightly brushed before imaging to clear surfaces of debris. Specimens were kept damp with 70% ethanol during imaging to prevent damage from drying, and all specimens were sampled in a non-destructive manner. We collected and reconstructed patches of surface topography at dimensions of either 22 × 14 mm or 11 × 7 mm, depending on specimen size and shape. Each topographic reconstruction has over 18 million 3D points (5202 × 3565 pixels reconstructed into three dimensions).

MEASURING SCALE AND SURFACE MORPHOLOGY

We used images collected from gel-based profilometry to measure several morphological aspects of surfaces and scales from the midbody and posterior regions (Fig. 2). We measured seven variables from both regions: roughness, skew, kurtosis, scale area, scale aspect ratio, ctenii coverage and ctenii length [ctenii are small spines that often occur in interlocking fields at the posterior margins of scales (Roberts, 1993; Wainwright & Lauder, 2016)].

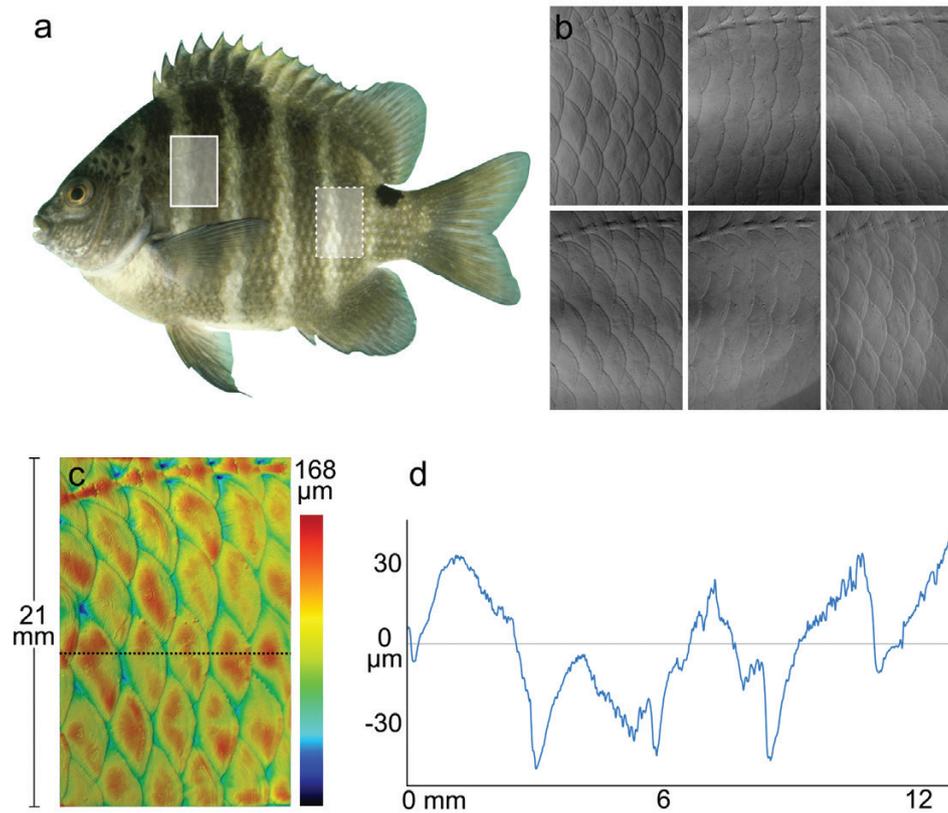


Figure 1. Sampling of damselfish surface topography. A, two sampling locations on the surface of damselfishes – one dorsal to the pectoral fin called midbody (shaded with solid line), and another anterior to the peduncle called posterior (shaded with dotted line). B, examples of black and white photographs collected during gel-based profilometry where lighting angle is changed, but the specimen remains fixed in position. C, three-dimensional surface reconstruction of the six images from B where each pixel is assigned a coordinate in 3D space. Warm colours refer to higher heights – height scale at right of image (darkest blue is 0 μm). D, example of a height profile line from the dotted line in C. The 0 μm height in this profile represents the mean height for the surface in C.

In this section, we describe measurement techniques and expected associations between these variables and function, when appropriate.

We use the 3D nature of our topographic data to measure three common and standard measurements of surface form (International Standard Organization, 2012): root-mean-squared roughness, skew and kurtosis. These measurements each provide different information about surface form and have been used in a series of other publications involving topographic investigation of biological surfaces (e.g. Wainwright & Lauder, 2018; Baeckens *et al.*, 2019; Wainwright *et al.*, 2019; Popp *et al.*, 2020). *Roughness* describes the variability in height across the examined portion of the body surface. We used topographic data to measure roughness as the root-mean-square of the difference between the height of each point on the surface and the mean height over the entire surface. Greater values of roughness indicate that the surface is more variable in height, while low values describe

uniform height across the surface. This measure of roughness is a common and fundamental parameter for describing surface characteristics (Whitehouse, 1994; International Standard Organization, 2012), and roughness variation may change the surface function as it interacts with surrounding fluid or other surfaces. In turbulent flow, for example, elements that increase roughness can decrease drag and sometimes increase thrust, as is seen in dimpled golf balls (Choi *et al.*, 2006), or shark denticle patterns (Oeffner & Lauder, 2012; Wen *et al.*, 2015). However, the fluid mechanisms that create drag reduction in these cases are not always understood, and the complexity of the fluid boundary layer makes it difficult to predict effects on drag. In laminar flow, however, smoother surfaces will decrease drag compared to rough surfaces (Smits, 2000). In addition to relationships between roughness and hydrodynamics, roughness may also affect other functions of fish scales such as physical protection or in helping to retain mucus and epidermis.

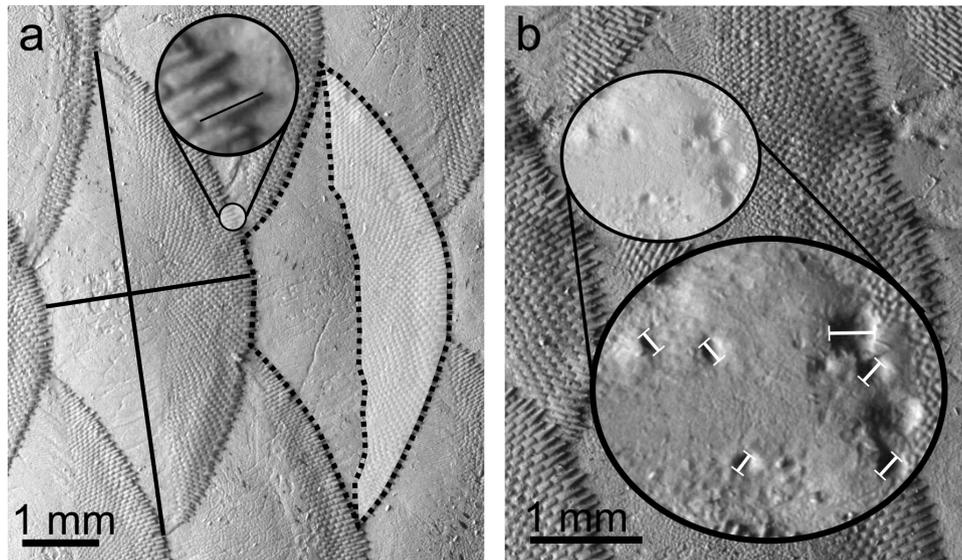


Figure 2. A, linear and areal morphological measurements taken from imaging and surface profilometry of scales. Solid intersecting lines indicate scale height and length, which are divided to give the aspect ratio. Dotted lines indicate scale area and the shaded area shows the area covered by ctenii. Ctenii coverage is the shaded area divided by the area enclosed by the dotted lines. Circular inset shows how ctenii length is measured. B, lateral line pore diameter.

We also make use of our topographical measurements to calculate skew and kurtosis of the distribution of heights from the fish body surface. *Skew* is a measure of the relative dominance of peaks or valleys on a surface (Whitehouse, 1994). Negative skew values indicate that valleys or other negative (hole-like) surface features are increasingly dominant, whereas positive skew values indicate the opposite – that peaks or other positive surface features dominate. *Kurtosis* tells us about the shape of the distribution of heights so that a normal distribution has a value of 3. In contrast, kurtosis values lower than 3 indicate a narrower distribution of heights with fewer values at low and high heights than expected, whereas kurtosis values above 3 indicate a wider distribution of heights and more extreme values than expected. Both skew and kurtosis give us additional morphological information about fish surfaces which we can study in a comparative context, though functional hypotheses make no predictions about how these properties should vary among species.

Our images of surface topography also allow us to measure a number of other aspects of scale morphology, including: scale area, scale aspect ratio, ctenii coverage and ctenii length.

Scale area is measured as the visible area of a scale (see Fig. 2) for three scales from each image. Misshapen and lateral line scales were avoided for these measurements.

Scale aspect ratio describes the shape of the exposed surface of the scale, not the entire scale (Wainwright & Lauder, 2016; Ankhelyi *et al.*, 2018). We measured

aspect ratio as the visible dorso-ventral height of the scale divided by the visible antero-posterior length (Fig. 2), and we quantified aspect ratio for each region of the body as the mean aspect ratio measured for three scales from that region. Aspect ratio is a simple measure of scale shape.

Ctenii coverage and *ctenii length* are the percentage area of the visible portion of the scale covered in ctenii and the mean length of individual ctenii on a scale, respectively. As described above, we estimated mean ctenii coverage and ctenii length for three scales from each body region (Fig. 2); for ctenii length in particular, three undamaged ctenii were measured per image, one each from three different scales. Ctenii are separate ossifications from the rest of the scale, which allows them to rotate at their base, so that their tips can point away from the body of the fish (Wainwright & Lauder, 2016; Spinner *et al.*, 2019). In some fish species, ctenii also protrude past the skin mucus (Wainwright & Lauder, 2018) and therefore could affect flows, perhaps by generating turbulence, organizing turbulence, or helping to maintain the epidermis and mucus coat (Wainwright & Lauder, 2016).

TRANSFORMATION OF VARIABLES AND SIZE CORRECTION

All variables were log-transformed and, if needed, corrected for variation due to specimen size. Damselfishes exhibit a relatively limited adult size range, and although we measured only adult specimens,

size variation is generally particular to our sample of specimens rather than a reflection of fixed differences in adult size among species. Across our sample, larger fish tend to have larger scales, and we sought to account for differences in trait values that could be explained simply by variation in size. In particular, we expected that roughness, scale area and ctenii length would have strong relationships with body size and would therefore need to be size-corrected at both midbody and posterior regions. To determine whether size correction was necessary for individual traits, we regressed log-transformed species mean trait values against log-transformed mean standard length (measured for each specimen prior to profilometry) while simultaneously accounting for phylogenetic signal in residuals (Revell, 2009, 2010). Regression models were estimated using the *phylolm* package (Ho & Ane, 2014) for R (R Development Core Team, 2018) under a lambda model (Pagel, 1999) given the consensus phylogenetic relationship estimated by Frédérick *et al.* (2013). We determined that a variable needed size correction when the *P*-value for the slope coefficient was below 0.05. According to this scheme, roughness, scale area, and ctenii length needed size correction but kurtosis, aspect ratio and percentage ctenii coverage did not. Surface skew showed a significant relationship with size at the posterior region, but we chose against size-correction for this variable because size explained only a small proportion of its variation and because skew exhibited a non-significant relationship with size for the midbody region. When size-correction was deemed necessary, we obtained size-corrected species values as the residual deviations from estimated regression lines. Using this method, residuals retain their phylogenetic signal (Revell 2009). Our choice to apply size-correction only to variables that had significant relationships with body size led to an interspecific data set that contains a mix of residuals and raw measurements. However, variables that were not size-corrected exhibited interspecific allometric slopes near zero, and taking residuals from the estimated regression would have little effect on variability among species values. We provide raw data as well as species' means for midbody and posterior regions in the [Supporting Information \(Tables S1 and S2\)](#).

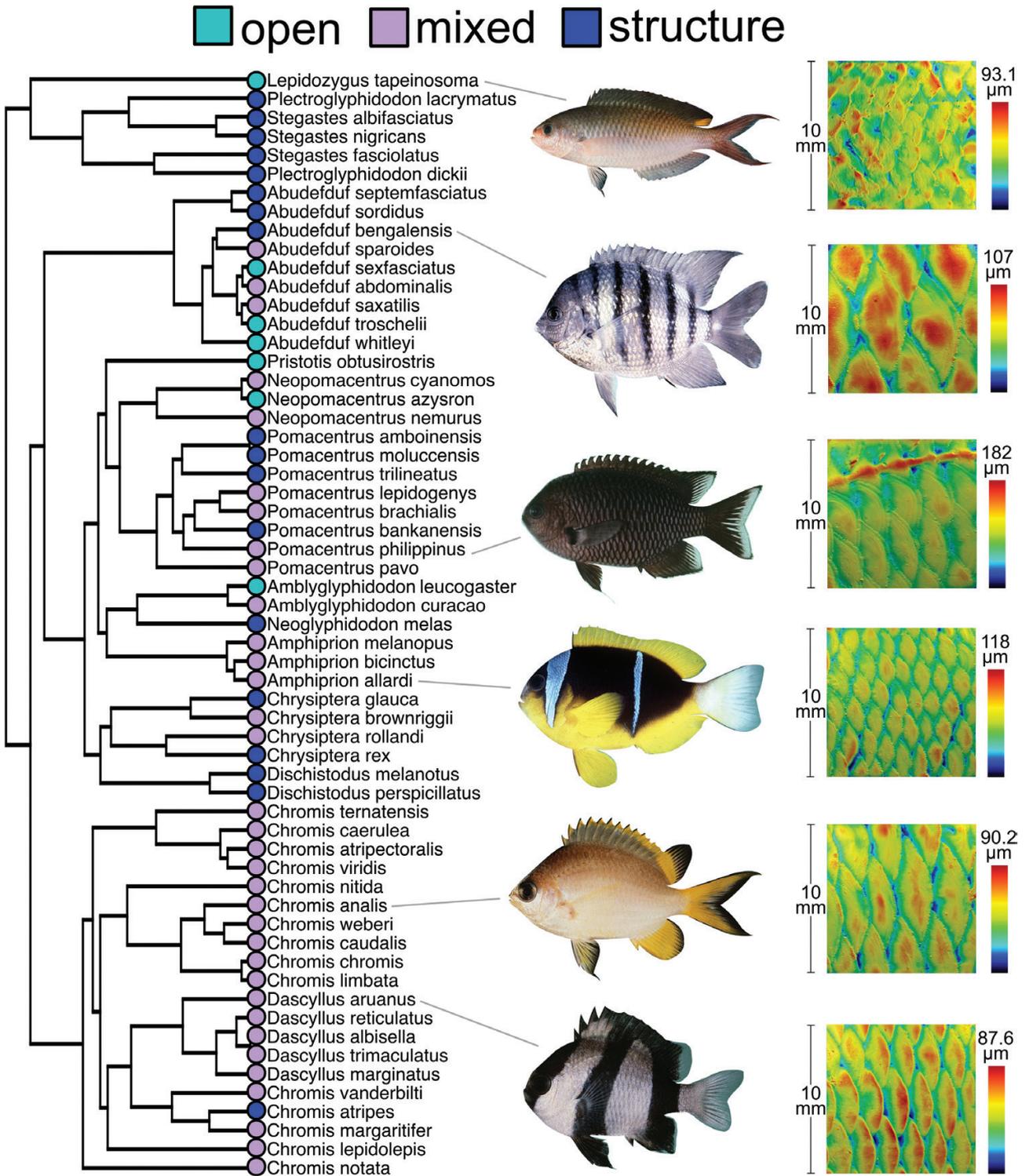
METHODS FOR AIM 1: WHAT FACTORS AFFECT SCALE FORM?

Our goal in aim 1 is to explore what factors are related to diversity in scale morphology. In particular, we narrow this aim into three questions: (1) does flow ecology affect scale morphology, (2) do shifts in the evolutionary optima of scales align with other ecological transitions, and (3) how are scale form and body shape related?

In question 1, we sought to understand how differences in the flows experienced by species might influence adaptive evolution of scale morphology. Although this connection between flow ecology and scale morphology has not been formally tested, experimental work suggests that scales can improve the hydrodynamic performance of fishes (Burdak, 1986; Wu *et al.*, 2018; Muthuramalingam *et al.*, 2019). To explore this idea, we created ecological categories that reflect the general feeding environment of a species by taking advantage of a well-studied axis between open-water and structure-associated feeding in reef fishes that also occurs within damselfishes (Findley & Findley, 2001; Wainwright *et al.*, 2002; Cooper & Westneat, 2009; Price *et al.*, 2012; Friedman *et al.*, 2016). We modified feeding-related categories used in previous studies of damselfishes that are based on prey type (e.g. zooplankton vs. benthic algae; Frédérick *et al.* 2013) by also considering how close to structure they tend to feed. We used ecological information from a variety of sources (Allen, 1975, 1991; Randall *et al.*, 1990; Froese & Pauly, 2018) and adopted three different categories: (1) open-water feeders that spend significant time in the water column that is adjacent to a reef or well above reef structures, (2) mixed feeders that capture prey about 1–3 m above reef structures, and (3) structure-associated feeders that primarily pick food near the benthos or feed from reef structures.

These three categories are meant to capture the flow regimes damselfish experience in their environments. Fishes feeding in open water tend to be exposed to direct currents and laminar flow (Odum & Odum, 1955; Hobson & Chess, 1978; Lazzaro, 1987; Hobson, 1991; Aguilar-Medrano *et al.*, 2016; Friedman *et al.*, 2016), and structure-associated feeders live in shallower water where large-scale turbulence and unsteady flows dominate (Fulton & Bellwood, 2005; Fulton *et al.*, 2005; Madin *et al.*, 2006; Reidenbach *et al.*, 2006; Koehl *et al.*, 2007; Aguilar-Medrano *et al.*, 2016). Our categorizations are shown in [Figure 3](#) and in [Supporting Information, Table S2](#). We refer to these different ecologies as open, mixed and structure for brevity.

To test relationships between flow ecology and scale morphology, we first applied permutation-based phylogenetic multivariate analysis of variance (pMANOVA). This analysis identifies linear combinations of scale variables that best separate species by flow environment categories and evaluates the significance of separation while controlling for phylogeny (Adams & Collyer, 2018, 2019). We included our multivariate dataset of 14 scale morphological measurements (seven variables at both midbody and posterior regions: roughness, skew, kurtosis, scale area, aspect ratio, ctenii coverage, ctenii length) and carried out pMANOVA by first scaling the data (dividing each



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Figure 3. Pruned consensus tree from [Frédérich *et al.* \(2013\)](#) including all species sampled in this study with tips coloured according to flow ecology. Grey lines indicate species photographs shown (photos from [Randall, 2020](#)). Example topographic maps of scaled surfaces are given for the pictured species at right. Note that each topographic image has a different height scale.

variable by its standard deviation), and then using the damselfish phylogeny of [Frederich *et al.* \(2013\)](#) and the function ‘procD.pgls’ in the geomorph and RRPP packages ([Collyer & Adams, 2018, 2021](#); [Adams *et al.*, 2021](#); [Baken *et al.*, 2021](#)) for R ([R Development Core Team, 2018](#)). We also tested the relationships between flow environment and individual morphological variables using permutation-based phylogenetic ANOVA (pANOVA). A general hypothesis for these analyses is that smoother surfaces would probably be beneficial for higher-performance swimmers living in unidirectional laminar flow ([Smits, 2000](#)), although our limited understanding of the fluid boundary layer that surrounds fishes prohibits any large degree of confidence in this hypothesis ([Anderson *et al.*, 2001](#); [Yanase & Saarenrinne, 2015](#)).

In question 2, we further evaluated the evidence for ecologically associated scale evolution by fitting multiple-optimum Ornstein–Uhlenbeck (OU) evolutionary models ([Hansen, 1997](#); [Butler & King, 2004](#)) to scale-morphological variables and the damselfish phylogeny. We applied the phylogenetic lasso method of the l1ou package and the phylogenetic Bayesian information criterion (pBIC; [Khabbazian *et al.*, 2016](#)) to identify shifts in adaptive optima on phylogenetic branches in the absence of a priori hypotheses about where these shifts occur. This method considered a large sample of multipeak OU models and therefore allowed us to evaluate support for the hypothesis that flow regimes are associated with distinct morphological optima alongside alternative optima configurations that may be related to other aspects of ecology or species biology. We applied the l1ou function ‘estimate_shift_configuration’ separately to each of our 14 variables using the [Frederich *et al.* \(2013\)](#) damselfish phylogeny. For each variable, we report the best-fit model (indicated by the lowest pBIC score) and alternative plausible models with comparable fit (i.e. models with pBIC scores within 4 of the best-fit model). We examined best-fit and plausible alternative models to assess the evidence supporting adaptive peak shifts associated with flow regime and to identify shifts in scale optima that coincide with the evolution of novel ecologies or body forms.

Finally, in question 3, we also tested associations with evolutionary changes in body shape in the form of body elongation. Body elongation has been shown in many studies to be a major axis of diversity for bony fishes that is often related to other important axes of morphological and ecological diversity ([Claverie & Wainwright, 2014](#); [Collar *et al.*, 2016](#); [Price *et al.*, 2019](#); [Friedman *et al.*, 2020](#)). For example, elongation is often related to swimming performance and transitions to more open-water habitats, where more deep-bodied fishes are thought to be more adept at manoeuvring in complex environments and more fusiform fishes have

been shown to have higher swimming performance ([Webb, 1984](#); [Langerhans, 2008](#); [Gerry *et al.*, 2012](#); [Walker *et al.*, 2013](#)). We therefore used body shape as an additional representation of ecology; although we have carefully categorized species into different flow ecologies (open, mixed, structure; see above), categories are always an imperfect representation of how a fish may spend its time. Body elongation provides a continuous axis of morphological diversity that is also linked to ecological diversity, and tests of evolutionary correlations between body elongation and scale morphology provided an additional way to evaluate scale adaptation to ecology. We measured body elongation for each of the species in our dataset using lateral view photographs from collections made publicly available online by the Bishop Museum and Dr John Randall ([Randall, 2020](#)). We calculated body elongation by measuring fish standard length and dividing it by maximum body height – more elongate fishes thus have larger values. We used phylogenetic regressions between scale morphology and body elongation to separately evaluate the strength of the relationship between body shape and all 14 measurements of scale morphology. We used the ‘phylolm’ function with a lambda model from the phylolm package ([Ho & Ane, 2014](#)) in R v.4.0.2 ([R Development Core Team, 2018](#)) to calculate regressions and correlations.

METHODS FOR AIM 2: EVOLUTIONARY INTEGRATION IN SCALE MORPHOLOGY

We also made use of our multivariate dataset to estimate the evolutionary correlations of scale morphological variables to reveal the degree of evolutionary integration in scales. In particular, we examined correlations among different aspects of scale morphology, between midbody and posterior regions, and separately for lateral line pore measurements. To study these first two sets of correlations, we calculated an evolutionary correlation matrix for all 14 scale morphology variables under a lambda model that accounts for multivariate phylogenetic signal ([Pagel, 1999](#); [Freckleton *et al.*, 2002](#); [Revell, 2009](#)). To prepare the data for this process, we first removed species descended from lineages that experienced l1ou-inferred peak shifts for any of the 14 variables because these species probably experienced alternative adaptive regimes that could have altered relationships among scale morphological variables. Ideally, we would evaluate potential differences in these relationships, but small numbers of species within derived adaptive regimes (see Results) prevented this possibility. Instead, most species retain the ancestral adaptive regime for scale morphological variables, and this strategy allowed us to examine evolutionary patterns for most of our sampled damselfishes ($N = 49$ species

after removal of ten species with peak shifts). We estimated the evolutionary correlation matrix under a lambda model by first estimating the multivariate 'lambda' (Pagel, 1999) for the 14 scale morphological variables using the 'phyl.pca' function in the phytools package (Revell, 2012) and transforming phylogenetic branch lengths by this lambda estimate using 'rescale' in the geiger package (Harmon *et al.*, 2008; Pennell *et al.*, 2014). We then calculated the correlation matrix from the evolutionary variance–covariance matrix estimated from the rescaled phylogeny and species data using the ratematrix function of the geiger package (Harmon *et al.*, 2008; Pennell *et al.*, 2014) for R (R Development Core Team, 2018). This function calculates the evolutionary variance–covariance matrix using phylogenetic independent contrasts (Felsenstein, 1985; Revell *et al.*, 2007). From the resulting correlation matrix, we examined relationships between different aspects of scale morphology within body regions and between midbody and posterior regions for each scale variable.

We also specifically explored the relationship between aspects of lateral line pore morphology. The lateral line is an important sensory system for fishes that mechanically senses water flow through the use of neuromasts that occur both on the surface of the fish and inside canals on the head and body (van Netten & McHenry, 2013; Carrillo *et al.*, 2019; Sato *et al.*, 2019). The lateral line trunk canal is typically a single canal that runs along the side of the body, and it houses sensory canal neuromasts and allows fishes to sense differences in pressure across their bodies. The trunk lateral line canal is made of modified scales that form a bony canal with pores that open to the external fluid environment, allowing pressure differences between pores at the skin's surface to be transmitted to the neuromasts housed inside the canal. Pores can occur at the margins between scales, but in some fishes (including damselfishes) the pores are in the scale itself and can lead directly to the canal and to smaller branches of the canal (Webb & Ramsay, 2017; Voronina & Hughes, 2018).

Variation in lateral line scales and the morphology of pores has been described and some authors have shown that different pore sizes and branching patterns can change how pressure signals are filtered before they reach the neuromasts inside the canals (Klein *et al.*, 2013; Klein & Bleckmann, 2015). Generally, models of lateral line canal function have shown that the diameter of canals serves as a low-pass filter to cut off high-frequency signals, and it is reasonable to hypothesize that pores function in a similar way (Denton & Gray, 1988). Despite some functional knowledge of how the lateral line canal operates, we still lack quantitative morphological data on lateral line scale diversity and its functional consequences.

Our data provide a prime opportunity to examine the evolution of quantitative lateral line diversity by focusing on pore morphology as proximal surface features of the lateral line canal.

We measured the number of pores per lateral line scale and the average pore diameter by using the images generated from profilometry at the midbody region, which always included part of the lateral line trunk canal (Fig. 2B). We chose these measurements because they were accessible and both the number of pores and the size of those pores have previously been shown to affect lateral line performance in artificial lateral line models (Klein *et al.*, 2013; Klein & Bleckmann, 2015). The number of pores per lateral line scale was counted on 3–5 scales per individual and the pore diameter was measured on 5–10 pores per individual. Means were then calculated for each measurement to represent individuals, and both measurements were size-corrected using the methods above. One important caveat to our measurements is that we recognized anything pore-like in our profilometry images to be a true pore, though it is possible that some of these pores could be other features such as depressions where superficial neuromasts occur on lateral line scales (Sato *et al.*, 2017).

We explored the evolution of pore morphology in damselfishes by examining the strength of the relationship between pores per scale and pore diameter in a phylogenetic context. To do so, we used the 'phylolm' function and a lambda model in the phylolm package (Ho & Ane, 2014) in R. This allowed us to calculate both the correlation between these two variables and the statistical significance of their relationship, marking the first time that trunk lateral line pores have been examined in a quantitative and comparative context.

RESULTS

RESULTS FOR AIM 1: ECOLOGICAL FACTORS THAT AFFECT SCALE FORM

We used a pMANOVA with all 14 of our scale-morphology variables in concert with our flow-related categories (open, mixed and structure) to test if scale and surface morphology are different in species that experience different flow environments. Our results show that scale morphology differs in species from different flow ecologies (pMANOVA, $F_{2,56} = 2.132$, $Z = 2.269$, $P = 0.012$). Results from subsequent single-variable pANOVAs show four of our 14 variables have a P -values less than 0.05: midbody roughness ($F_{2,56} = 7.07$, $P = 0.0024$), midbody ctenii coverage ($F_{2,56} = 4.515$, $P = 0.0157$), posterior roughness ($F_{2,56} = 3.1605$, $P = 0.0484$) and posterior scale aspect ratio ($F_{2,56} = 4.8206$, $P = 0.0138$). The open-water ecology

group has lower midbody and posterior roughness, higher ctenii coverage, and lower scale aspect ratio compared to either the mixed and structure flow-ecology groups (details depend on variable; Fig. 4). Results of pANOVAs from all 14 variables are given in the [Supporting Information](#).

After exploring connections between flow ecology and scale morphology, we then used *l1ou* to search for transitions in adaptive peaks for scale morphology across our sampled species. The best-fit multipeak OU models by *l1ou* generally inferred few peak shifts for most variables. Of the 14 variables, five had best-fit models with zero shifts in trait value across our sampled species: midbody roughness, midbody skew, midbody and posterior kurtosis, and midbody aspect ratio. Six variables showed a single shift according to *l1ou*: posterior roughness, midbody and posterior ctenii coverage, posterior scale aspect ratio, and midbody and posterior ctenii length. The best-fit model for posterior roughness inferred a transition to higher roughness along the internal branch that represents the common ancestor of the genus *Dascyllus* (Fig. 5; represented in our dataset by *D. aruanus*, *D. reticulatus*, *D. albisella*, *D. trimaculatus* and *D. marginatus*). Both midbody and posterior ctenii coverage showed a single transition to a lower value on the terminal branch for *D. aruanus*. For posterior aspect ratio, we identified a single transition to more elongate scales along the terminal branch for *Lepidozygus tapeinosoma*. In addition, both midbody and posterior ctenii length showed a single peak shift towards shorter ctenii lengths, but that

transition occurs at slightly different locations; for midbody ctenii length, we found a transition on the internal branch that subtends *Amphiprion allardi* and *Amphiprion bicinctus*, whereas for posterior ctenii length we found a transition at the internal branch that subtends all three species of *Amphiprion* included in this study: *A. allardi*, *A. bicinctus* and *A. melanopus*. There were two shifts toward reduced visible scale area for both midbody and posterior regions – one along the terminal edge for *Lepidozygus tapeinosoma* and another on the internal edge that subtends the genus *Amphiprion* (represented by *A. allardi*, *A. bicinctus* and *A. melanopus*). Finally, the best-fit model for posterior skew inferred three transitions, all towards lower skew values (more valley-like features): along the terminal edges for *Abudefduf septemfasciatus* and for *Amphiprion allardi*, and along the internal edge that subtends *D. albisella* and *D. trimaculatus*. The complete results for this analysis are presented in [Supporting Information, Table S3](#), where we give the models of best fit (the best model and those within 4 pBIC units of the best model) for each of the 14 scale morphology variables that were analysed.

Examination of the phylogenetic location of *l1ou*-inferred peak shifts revealed little evidence that transitions in flow environment are associated with major shifts in scale morphology. We found that for only three of the morphological variables in our dataset, the best-fit multipeak OU models included peak shifts associated with a transition to open-water ecology ([Supporting Information, Table S3](#)). In all three cases (midbody and

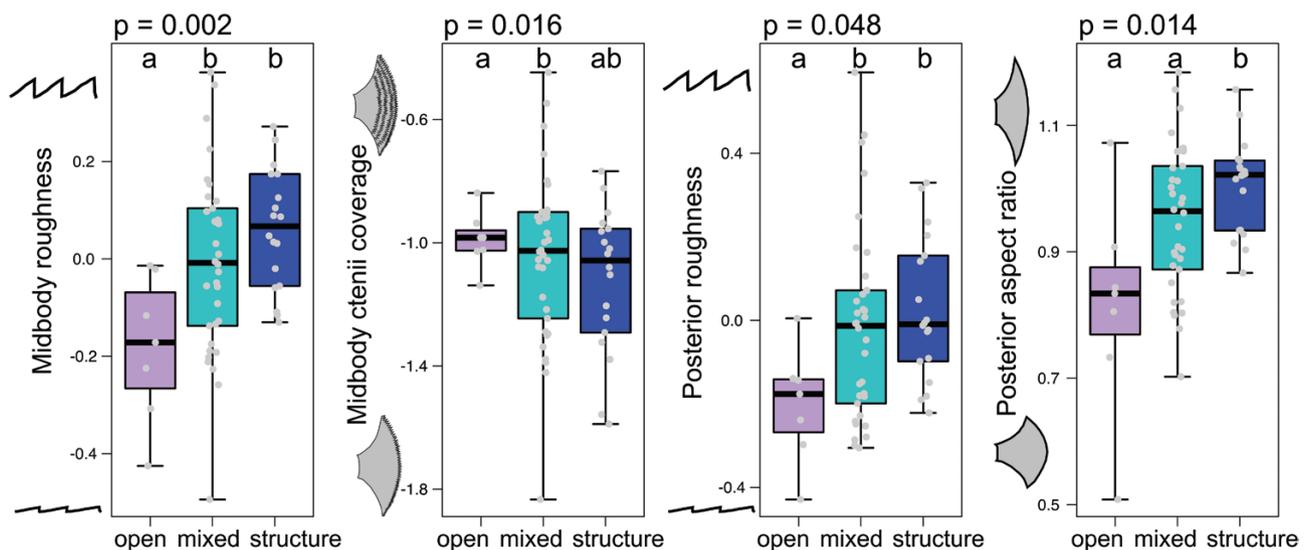


Figure 4. Differences in scale morphology among flow ecology categories. Data are presented as box and whisker plots with the thick bar representing the median, the box representing the interquartile range, and each whisker representing the remaining first and fourth quartiles. Grey points are species means of data that have been previously log-transformed and size-corrected (where needed). The provided *P*-values are from permutation-based ANOVA (see Methods and Results) and pairwise significant differences are indicated by lower-case letters.

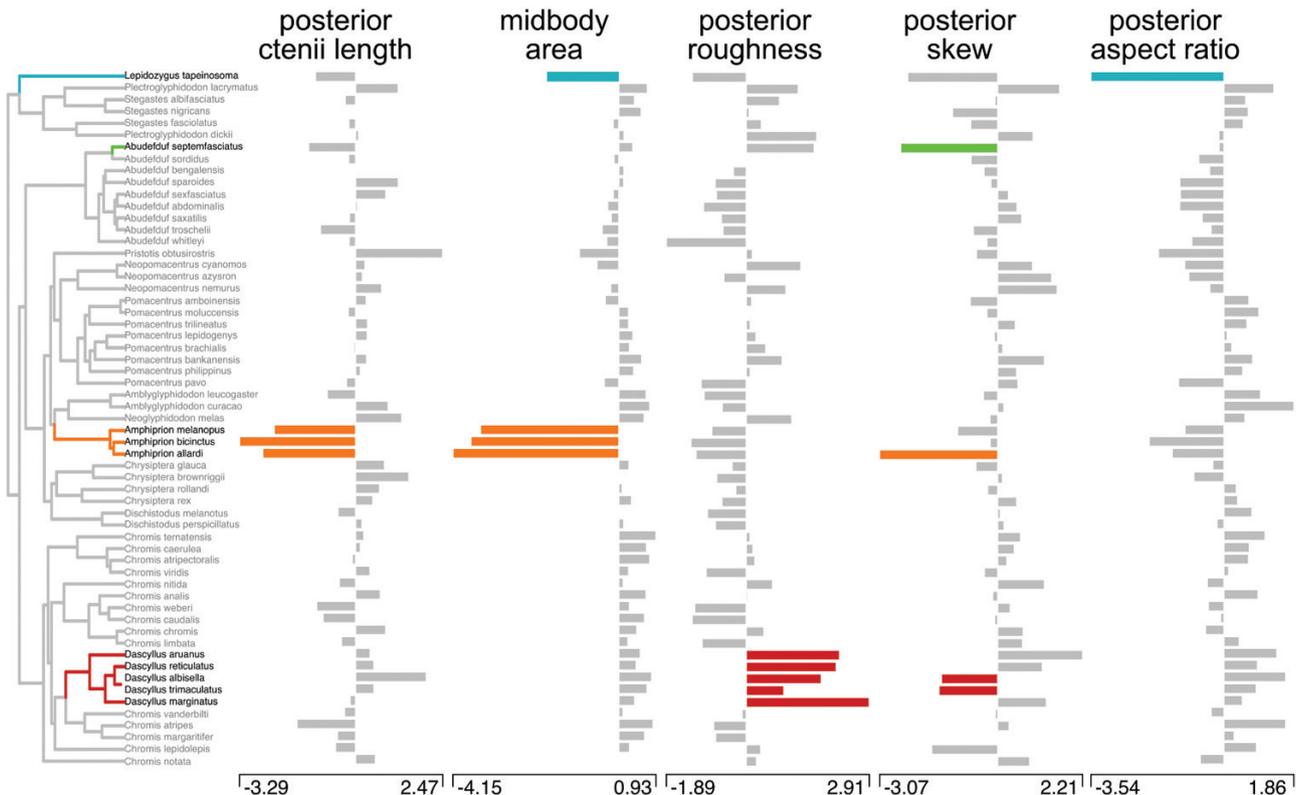


Figure 5. Selected results from 11ou analyses that show clades where multiple transitions in scale morphology have occurred alongside novel ecological or morphological diversity. Anemonefishes (*Amphiprion* spp.) live with anemones and show transitions in posterior ctenii length and midbody scale area (and also posterior scale area, not shown here). *Lepidozygus tapeinosoma* has an elongate body and open-water swimming lifestyle, and shows transitions in midbody (and posterior – not shown) scale area, and posterior aspect ratio. *Dascyllus* spp. are deep-bodied damselfishes that commonly form colonies in branching coral. They show transitions in posterior roughness, and smaller portions of their clade also show transitions in posterior skew (as well as midbody and posterior ctenii coverage – not shown here).

posterior area, posterior aspect ratio), these variables only showed a transition in a single open-ecology species – *Lepidozygus tapeinosoma* (Bleeker 1856) – and in two of those cases (midbody and posterior area) the best-fit model also estimates transitions in a similar direction for species that are not in the open-ecology category. While an additional six of the morphological variables had an alternative plausible model (within 4 pBIC units of the best fit; Table S3) that included a peak shift associated with a transition to open-water ecology, most of these were shifts in only a single open-ecology species (in either *L. tapeinosoma* or *Pristotis obtusirostris*). Overall, these results may initially seem at odds with our findings of differences in scale morphology based on flow ecology (using pMANOVA and pANOVA), but these are fundamentally different analyses. Instead, our results suggest that species inhabiting different flow environments tend to differ for some morphological variables, but these differences are minor compared to the more extreme lineage-specific changes that may be associated with other ecological transitions.

We also studied how body shape – in particular body elongation – affects scale morphology because body elongation is an important axis of fish body diversity and can be a predictor of ecology. Phylogenetic linear models showed that body shape has non-significant or significant but weakly explanatory relationships with most measures of scale morphology (Supporting Information, Table S4). For example, midbody roughness, midbody scale area and posterior kurtosis all have significant relationships with body shape ($P < 0.05$), with r^2 values between 0.08 and 0.13, which indicates that body shape explains just a small proportion of the variability in these traits. Another nine variables (posterior roughness, midbody and posterior skew, midbody kurtosis, posterior scale area, midbody and posterior ctenii coverage, and midbody and posterior ctenii length) exhibit non-significant relationships with body shape. In contrast to this general pattern, we find that both midbody and posterior aspect ratio have strongly significant and moderately explanatory relationships with body

shape, such that more elongate body shapes predict more elongate scale shapes (Fig. 6; body shape vs. midbody aspect ratio: adjusted $r^2 = 0.286$, $P < 0.0001$, lambda = 0.783; body shape vs. posterior aspect ratio: adjusted $r^2 = 0.357$, $P < 0.0001$, lambda = 0.941).

RESULTS FOR AIM 2: EVOLUTIONARY INTEGRATION IN SCALE MORPHOLOGY

Evolutionary correlations among different aspects of scale morphology were generally weak, with most pairs of variables having correlation coefficients between -0.4 and 0.4 (Fig. 7). One exception to this pattern was the correlation between scale area and aspect ratio where midbody scale area had positive correlations of 0.7 with both midbody and posterior scale aspect ratio, indicating that larger scales tend to be taller and less elongate in shape. Posterior scale area was also correlated with posterior aspect ratio (0.54), but more poorly correlated with midbody aspect ratio (0.23). Correlations between the same aspect of scale morphology at different body regions were generally strong (0.6 – 0.85) with the exception of skew and kurtosis, which both had weaker correlations of 0.37 (Fig. 7). Note that evolutionary correlations were estimated following removal of the 10 species that were inferred by I1ou to be in derived adaptive regimes (see above) and transformation of phylogenetic branch lengths by the empirical multivariate lambda = 0.39 . In our analyses, any correlations with magnitude greater than 0.282 are significant at $P = 0.05$ ($N = 49$, d.f. = 47).

We used a phylogenetic linear model to examine the relationship between the number of pores per lateral line scale and the average diameter of those pores. We found a significant relationship between these two

variables, such that increasing the number of pores per lateral line scale predicts a smaller diameter for the pores (Fig. 8; adjusted $r^2 = 0.385$, $P < 0.0001$, Pearson's correlation coefficient = 0.62 , lambda = 1).

DISCUSSION

Scales are common to the majority of fishes and exhibit a diversity of forms that has inspired hundreds of years of study. The factors that shape this diversity, however, remain incompletely understood in part because of a lack of phylogenetically informed investigations of quantitative scale morphology (but see Viertler *et al.*, 2021). Moreover, until recently we have lacked easy methods for imaging and measuring scale morphology in three dimensions even though surface characteristics are crucial to understanding how fish interact with the fluid environments. Our study sought to fill these gaps by presenting a dataset that describes multiple aspects of 3D scale structure in a diverse clade of fishes. Overall, we found support for an hypothesized association between flow ecology and scale morphology in damselfishes; scales are hypothesized to influence interactions between ambient flows and the fish body surface (Burdak, 1986; Wainwright & Lauder, 2016; Muthuramalingam *et al.*, 2019). While flow environment appears to have an effect on scale form, in most cases transitions in flow ecology do not align with shifts in adaptive peaks for aspects of scale morphology. Instead, inferred peak shifts are associated with other novel lifestyles and body forms (anemone-living, deep or elongate bodies, etc.). These results suggest that flow environment does influence at least some aspects of scale morphology,

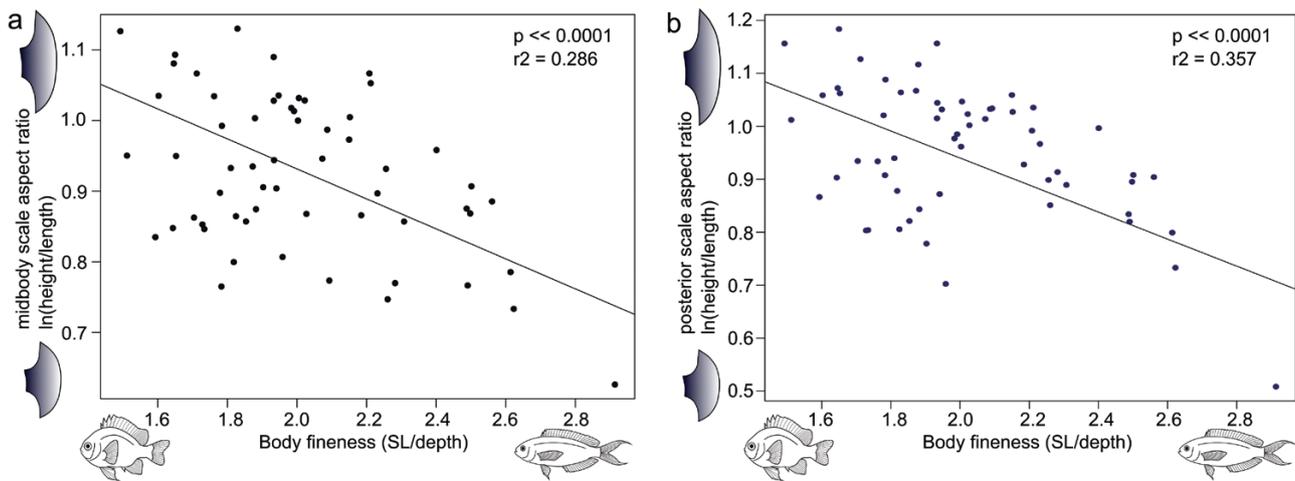


Figure 6. A, body shape (body fineness: standard length divided by maximum body depth) vs. midbody scale shape (visible aspect ratio: height divided by length; Fig. 2). B, body shape vs. posterior scale shape. P -value and r^2 are from phylogenetic linear models (see Methods).

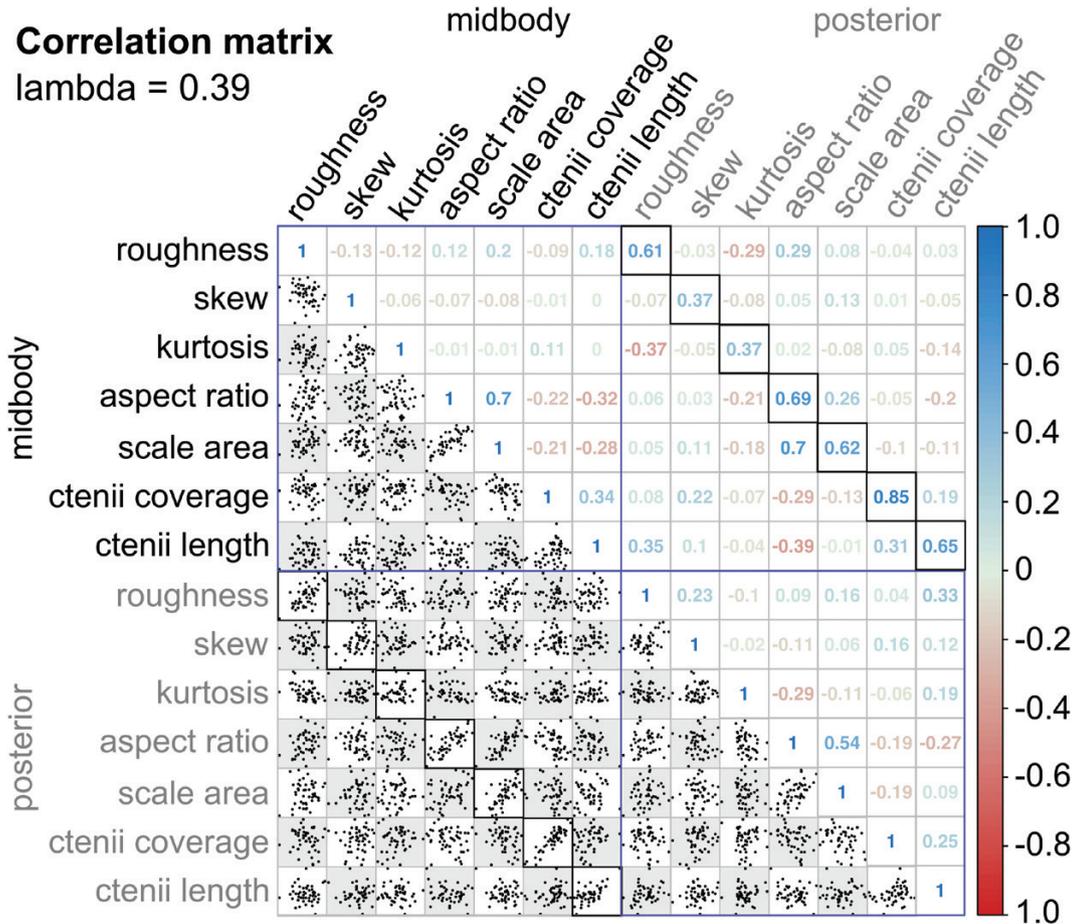


Figure 7. Correlation and scatterplot matrix of all 14 measurements of scale morphology. Evolutionary correlations from phylogenetic independent contrasts and a lambda model of evolution. Large blue boxes are correlations within a body region. Boxes outlined in black are correlations between midbody and posterior measurements of the same variable. Any correlations stronger than 0.282 (or -0.282) will be significant at a *P*-value of 0.05 (*N* = 49, d.f. = 47).

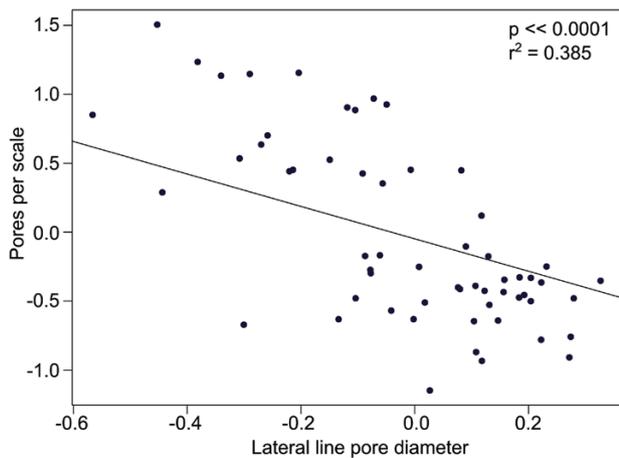


Figure 8. Pores per lateral line scale vs. pore diameter. *P*-value and *r*² are from a phylogenetic linear model (see Methods).

but major evolutionary changes in scales are driven by other ecological factors. In addition, body elongation is strongly related to scale shape, demonstrating that this canonical measurement of fish diversity (body elongation) may help explain one aspect of scale variability. Our results also show that, in general, aspects of scale morphology tend to be weakly evolutionarily integrated in damselfishes, though morphological features exhibit significant integration between body locations and there is a negative evolutionary relationship between the number and size of pores in the lateral line scales. Altogether, our results provide new insights into the evolutionary factors that shape the diversity of fish scales.

SCALE MORPHOLOGY AND FLOW ECOLOGY

Our use of pMANOVA and pANOVA found associations between flow-related ecology and scale morphology in

damsel-fishes. These findings support the hypothesis that fluid interactions with the skin's surface exert a consistent influence on scale surface properties and shape in this group. In particular, we demonstrate that species in the open category have lower midbody and posterior roughness, higher midbody ctenii coverage, and lower posterior aspect-ratio scales (less 'tall' and more elongate in an antero-posterior direction) compared to either the mixed or structure category (depending on the variable; Fig. 4). Despite the detection of these differences, we do not find a strong association between transitions in flow ecology and shifts in adaptive peaks; just a single open-ecology species (*L. tapeinosoma*) is associated with adaptive peak shifts. At first the results from these two analyses (pANOVA vs. modelling shifts in adaptive peaks) seem contradictory, but they are testing different questions; pANOVA evaluates whether scale morphologies tend to differ among species that inhabit different flow regimes, whereas multi-peak OU models identify major morphological shifts. Our combined results suggest that variation in flow ecology can drive scale diversity, but the overlap in scale morphology between flow regimes (Fig. 4) and the prevalence of adaptive peak shifts unrelated to flow ecology shifts (Fig. 5) indicate that other factors may be more important for generating more extreme scale morphologies in damselfishes (see also discussion of adaptive peak shifts below).

Nonetheless, our analyses do show evidence of differences in scale morphology in different flow ecologies, in support of past morphological studies in other fish groups that have hypothesized flow-related functions for scales (Fletcher *et al.*, 2014; Wainwright & Lauder, 2016), and experimental studies that have shown beneficial fluid effects of fish scales (Wu *et al.*, 2018; Muthuramalingam *et al.*, 2019). In particular we find that the open category fishes, which spend more time swimming in the water column, have smoother midbody and posterior surfaces, which supports our hypothesis that smoother surfaces may have increased hydrodynamic benefits (e.g. drag reduction) in species that spend more time steady-swimming in unidirectional laminar flow. Generally in fluid dynamics, smoother surfaces help to minimize drag caused by the boundary layer by allowing the boundary layer to remain laminar and preventing separation from the surface, which would incur a large drag penalty. Although a turbulent boundary layer can better prevent separation, they are typically of higher drag than laminar ones (Smits, 2000).

We also find that midbody ctenii coverage is higher in the open-ecology compared to the mixed-ecology group (with the structure category indistinct from either but with values closer to the mixed category). This confirms

a similar result found using different methods in cichlids, where ctenii coverage was found to be weakly related to stable isotope signatures, a proxy for ecology (Viertler *et al.*, 2021). Ctenii are small spines that can occur on the posterior edge of scales, and authors have previously hypothesized that ctenii may increase hydrodynamic and swimming performance through a number of potential mechanisms, such as: preventing backflow and separation by passively pivoting up during backflow events, organizing turbulence to decrease drag, enhancing epidermis and mucus attachment to the surface (mucus probably decreases drag), creating turbulence to prevent flow separation, or decreasing skin stiffness as scales contact each other during skin and body bending (Hoyt, 1974; Daniel, 1981; Burdak, 1986; Wainwright & Lauder, 2016, 2018; Spinner *et al.*, 2019). Although our methods are unable to distinguish any of these hypotheses, our results suggest that the degree of ctenii coverage may indeed play a role in hydrodynamics.

We further show that species in the open category have more elongate scales (in an antero-posterior direction) compared to those in the structure category. This association may reflect a linkage between body shape and scale shape (see discussion of body shape below) and a tendency for species in the open category to have more elongate bodies and therefore have more elongate scales (body elongation is often associated with open-water ecologies; Friedman *et al.*, 2020). In contrast, a recent study (Viertler *et al.*, 2021) looked at whole scale shape in the Tanganyikan radiation of cichlids and did not find a link between whole shape and measures of ecology (stable isotope values and body shape), although the authors discuss that their ecological axes may not fully capture the environmental factors that influence scale morphology (e.g. cichlid body elongation occurs in multiple different habitats). Regardless, scale shape is a major axis of scale diversity (Roberts, 1993; Ibañez *et al.*, 2009; Viertler *et al.*, 2021), and our analyses reveal a link between scale shape and flow environment that may be driven by direct effects of flows or by constraints imposed by body shape. As with most scale characteristics, functional studies of scale shape are lacking, although scale shape may influence material properties such as stiffness and puncture resistance of the skin, and may also change boundary layer flows – more work is needed to test these ideas.

Although we have confidence that our ecological categories represent meaningful differences in flow environments, we recognize the limitations of categorizing a complex ecological axis that would probably be better represented as a continuum. Ideally, we would have information on both the swimming behaviour of species in wild habitats (e.g. tail-beat frequency, average speed, turn frequency

and angle), microhabitat occurrence, and the natural flow patterns that occur in various reef habitats and microhabitats. However, comparative datasets on reef flows, microhabitat occurrence and swimming performance are rare in fishes. These data are difficult and resource-intensive to collect but would provide a more accurate representation of how these species interact with the fluid environment.

BODY SHAPE AND SCALES

Body shape is recognized as an important component of fish diversity and we have a growing understanding of how body shapes change with ecology and habitat (Webb, 1975; Walker *et al.*, 2013; Friedman *et al.*, 2020). Furthermore, we know that body elongation is an important axis of body shape diversity for fishes (Claverie & Wainwright, 2014; Price *et al.*, 2019) with ecological consequences that are typically aligned with swimming performance (Webb, 1984; Langerhans, 2008; Gerry *et al.*, 2012; Walker *et al.*, 2013). We therefore sought to examine associations between body elongation and scale shape. Overall, we found that body elongation is significantly and strongly related to both midbody and posterior scale shape (aspect ratio) where more elongate bodied fishes possess more elongate scales (Fig. 6). Our study is the first that we are aware of to document this pattern, and it ties the functionally important and well-studied axis of body shape diversity to the more poorly understood axis of scale shape. Scale shape probably has an effect on puncture and deformation resistance, and it could also influence boundary layer fluid dynamics; however, more functional studies are needed to test these connections between scale form and function. We also find significant but weakly explanatory relationships between body elongation and midbody roughness, midbody scale area and posterior kurtosis, suggesting that ecology in the form of body shape also influences additional measures of scale morphology, albeit more weakly than it does with scale shape.

ADAPTIVE PEAK SHIFTS IN SCALE MORPHOLOGY

Overall, we found few transitions in adaptive regime for scale morphology across damselfishes, with 11 of 14 morphological variables showing zero or one adaptive peak shifts across our sample of species. However, inferred peak shifts do suggest a role for ecology and ecomorphology in shaping scale morphology. An example is seen in the anemonefish genus *Amphiprion*, where we find that anemonefish scales transition to smaller size and smaller ctenii (spines), and one species (*A. allardi*) also shows a transition to a lower skew, indicating more valley-like features on the surface

(Fig. 5). Perhaps these smaller scales, spines and lower skew all help make surface features less prominent as anemonefishes build up important mucus layers that help them avoid being stung by their host anemone's nematocysts (Mebs, 2009). Regardless, these results provide evidence that shifts in ecology might be linked to shifts in scale and surface morphology.

We also find shifts in scale morphology in *L. tapeinosoma*, which spends more time in open water than other damselfishes (Allen, 1975, 1991). This species also exhibits the most elongated body shape of any species in our dataset, indicating a potential for higher sustained swimming performance. Along with these transitions in habitat use and body shape, this species experienced peak shifts towards smaller scale sizes at the midbody and posterior regions as well as more elongate posterior scales, which is consistent with the positive body and scale shape correlation we found for other damselfishes (see discussion of body shape above) though more extreme. The transition to smaller scales in *L. tapeinosoma* perhaps reflects decreased investment in physical protection as its more elongated body shape may confer higher swimming and escape performance in open habitats, as has been documented in other fishes (Webb, 1975; Friedman *et al.*, 2020).

In addition, we found shifts in scale morphology in *Dascyllus*, including a shift to a higher posterior surface roughness for all sampled species in the genus as well as shifts to a lower ctenii coverage for both the midbody and posterior regions for *D. aruanus*. As a genus, *Dascyllus* species tend to be planktivorous, deep-bodied and associated with branching corals, which are used as shelter (Allen, 1991; Frédérick *et al.*, 2013). The functional significance of higher posterior surface roughness in this group is not clear, but perhaps a rougher surface helps resist abrasion that occurs while associating with branching corals.

We believe these results demonstrate potential instances where scale morphology changes in response to the evolution of new ecologies or body shapes, such as living in anemones (*Amphiprion*), open-water specialization and body elongation (*L. tapeinosoma*), or coral association and the origin of an especially deep-bodied morphology (*Dascyllus*). Although we are unsure of the functional implications for these changes in scale morphology, we believe that documenting these transitions and their potential associations with derived ecologies and body shapes is an important step in understanding the generation of scale diversity. Furthermore, these results demonstrate that ecological axes beyond flow environment may be responsible for transitions to more extreme scale morphologies in this group, and other ecological considerations, such as associations with anemones or corals, may be strong drivers of scale evolution.

EVOLUTIONARY INTEGRATION OF SCALE MORPHOLOGY

In an attempt to better understand evolutionary integration of different aspects of scale morphology, we investigated the correlations of our suite of morphological measurements. Our results show that most aspects of scale morphology are only weakly correlated with one another (Fig. 7). This pattern suggests that these different structural features are able to vary largely independently from one another, potentially indicating that scales have a number of evolutionary axes that can contribute to the large diversity of scale forms we see across fishes, such as in many-to-one mapping of form to function (Wainwright, 2007).

Although most variables were not strongly correlated, scale shape (aspect ratio) and size (visible area) exhibit a relatively strong correlation at both midbody and posterior sites, indicating that larger scale areas correspond to taller and less elongate scales in damselfishes. This relationship may reflect functional constraints on damselfish scale morphology in which scale size and shape interact to affect body flexibility during lateral bending; large scales that are elongate may be functionally disadvantageous because they bunch up and inhibit body bending (Szewciw *et al.*, 2017; Shafiei *et al.*, 2021). Future studies could examine how scale shape and size are related in other groups to confirm this pattern, and experiments or comparative studies could seek to reveal potential functional or developmental reasons behind this relationship.

EVOLUTIONARY INTEGRATION OF LATERAL LINE PORE MORPHOLOGY

In lateral line canals, flow signals in the form of pressure are transmitted from the outside environment through pores and into the lateral line canal. If there are differences in pressure between sets of pores, the fluid inside the canal will move towards regions of low pressure and neuromasts will detect these changes with hair cells embedded in a gelatinous cupula that projects into the canal. Our general understanding of how the lateral line canal operates is firm, but we have yet to fully tie the large diversity of lateral line canal scales (Voronina & Hughes, 2018) with functional diversity or tradeoffs. Furthermore, recent work has demonstrated that many past representations of lateral line canal pores failed to accurately depict pore morphology (Webb & Ramsay, 2017), and lateral line pores are not commonly considered in biomathematical models of lateral line canal function. However, by building on excellent models of canal function (Denton & Gray, 1988; van Netten & McHenry, 2013) and using experiments with physical models of various lateral

line pore configurations (Klein *et al.*, 2013; Klein & Bleckmann, 2015), we do know that pore size, spacing and branching configuration are important to lateral line function. In particular, canal and pore diameter are thought to function as low-pass filters for flow signals (Denton & Gray, 1983). In general, smaller pores are thought to have a number of different effects on lateral line function, as they probably increase the flow resistance (therefore decreasing amplitude sensitivity), create higher cut-off frequencies (thus increasing the permissible range of frequencies as a low-pass filter) and decrease the spatial sensitivity of lateral line canals (Klein *et al.*, 2013; Klein & Bleckmann, 2015). However, having a network of pores appears to increase signal to noise ratio when environmental noise such as bubbles is present (Klein *et al.*, 2013; Klein & Bleckmann, 2015).

We found that the number of pores per scale has a significant inverse relationship with the size of the pores, where having more pores per scale is associated with smaller pores, and having fewer pores is associated with larger pores. This relationship might represent the functional requirement of keeping flow resistance constant – multiple small pores can provide the same resistance as a few larger pores – while adapting to the demands of detecting pressure fields in different flow environments. Many small pores may confer increased signal-to-noise in a noisy environment, whereas fewer large pores probably provide better spatial resolution and sensitivity. However, many other factors that have not been measured here (e.g. canal, tubule and cupula sizes) also contribute to lateral line performance, and thus we are limited in our hypotheses of function. Nonetheless, our study demonstrates an axis of diversity in lateral line pore morphology that may represent diversity in function, revealing the potential for studying lateral line pore configurations more closely in the future.

CONCLUSION

In summary, our results demonstrate a mixed set of answers to the long-standing question of why fish scales are so diverse. We show that some changes in general ecology coincide with shifts in scale form in damselfishes, and that both flow ecology and body elongation are associated with differences in scale form, providing evidence that ecologically driven selection could underlie scale diversity. Additionally, while scale morphology tends to be correlated between body regions, different measurements of scale morphology are not often related, with the exception of scale size and shape. Perhaps the general lack of evolutionary integration between aspects of scale morphology shows us that scale form is evolutionarily labile and that not

all the parameters we measured affect function or are predictably related to function. We also discover that if a species has more lateral line pores per scale, those pores tend to be smaller, which may reflect an attempt to keep a consistent hydrodynamic signal into the canal while trading-off between performance in noisy environments and spatial sensitivity. Future work that examines other clades of fishes in a similar manner will help place our results in a broader context and determine the generalizability of our conclusions.

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DATA AVAILABILITY

All data used in this paper are available as Supporting Information in [Tables S1 and S2](#).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Individual values.

Table S2. Species means.

Table S3. Model results.

Table S4. Body elongation (shape) vs. scale morphology.