Epidermal microstructures on the paired fins of marine sculpins provide new insights into mechanisms supporting benthic station-holding

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Abstract

Harsh environments, such as those with breaking waves and turbulent flows, present extreme challenges to organismal survival. Many animals exploiting these habitats possess adaptations to maintain position under dynamic flow conditions, such as reversible or permanent attachment systems. However, some station-holding fishes (e.g., sculpins) instead rely on morphological and behavioral modifications of their pectoral fins to increase friction with the substrate and combat drag. Despite epidermal microstructures on the fins of other benthic fishes, little exploration of pectoral fin surfaces at the microscopic scale has been undertaken in sculpins. Using scanning electron microscopy, we discovered microscopic, fibrillar projections contained within single cells on the ventral surfaces of the paired fin rays of two intertidal and two subtidal species of marine sculpins. In contrast to subtidal species, the intertidal species possessed epidermal cells with discrete channels separating groups of fibrillar projections. These features bear a striking resemblance to epidermal microstructures described in other fishes but have distinct morphological differences. We posit that these previously overlooked features likely contribute to sculpin station-holding performance via enhanced mechanical interactions with the substrate, suggesting new taxa within which to examine mechanisms of underwater friction enhancement and adhesion.

Introduction

Animals face many challenges in their environments, often possessing specialized morphological and functional adaptations to endure conditions that may otherwise limit survival. For example, aquatic environments with fast or turbulent flow can easily displace or dislodge animals and result in negative outcomes including predation, injury, and/or death. The monumental challenge of dealing with flow can thus act as an environmental filter on morphological traits, such that specific phenotypes are more common in more challenging environments [1–3]. Benthic animals subjected to strong hydrodynamic forces imposed by flow have often evolved discrete attachment systems, such as suction or adhesive-secreting discs, that help them maintain their position on aquatic substrates when exposed to flow [4–7]. However, attachment systems are not ubiquitous in turbulent environments, or even in benthic animals that maintain their position in flow. In fishes, for example, species without attachment systems maintain a stationary position (i.e., station-hold) by co-opting their body and fins to oppose or limit dislodgement forces [3,8–18]. Whole animal traits such as streamlined body shapes, small body size, and negative buoyancy are important contributors to this function, but the use of the pectoral fins, specifically, is often key to mitigating flow challenges through shape and position changes [8,13,15,17,19–21].

The pectoral fins of station-holding perciforms such as blennies (Family Blennidae), hawkfish (Family Cirrhitidae), scorpaenoid fishes such as sea robins, scorpionfishes, and rockfishes (Suborder Scorpaenoidei), and cottoid fishes including sculpins and poachers (Suborder Cottoidei) often possess a regionalized design [11,18,20,22–26]. The specialized ventral aspect of pectoral fins contacts the substrate similar to claws [18,23,24], while the more typical dorsal aspect is positioned to generate negative lift, pushing the fish toward the substrate [1,8,15]. Although the link between fin regionalization and station-holding in benthic fishes is strong, it is not required and other possible mechanisms may be employed. For example, rheophilic, freshwater Ostariophysian fishes lack regionalized fins but exhibit microscopic, cornified, often elongate epithelial projections on the ventral surfaces of their paired fins, termed ‘unculi’ [27,28]. These features are considered characteristic of this group and function in increasing friction and/or adhesion with the substrate [29], as well as

providing protection from mechanical abrasion or aid in proprioception and/or chemoreception [27,30]. Additionally, benthic darters can maintain position in flow simply by taking advantage of hydrodynamic interactions alone without the presence of regionalized fins or microscopic features [1,17,18,28]. Therefore, fishes likely employ a subset of possible traits to combat the forces imparted by fast or turbulent flows, and additional functional mechanisms may be present among station-holding fishes that have not been previously considered.

Despite the interest in morphological specialization for station-holding in sculpins, specifically [8,11,20,23–26,31–34], existing studies primarily focus on macroscopic characteristics that aid in position maintenance. Here we used scanning electron microscopy to describe intra- and interspecific variation in microscopic projections on the ventral surface of pectoral and pelvic fin pads of four species of marine sculpin. We find evidence of newly described, raised epidermal projections similar to those described in other fishes, suggesting that such features may be more broadly present in benthic fishes than what has been described previously.

Materials and methods

Three specimens from each of four species within the Superfamily Cottoidei were examined: intertidal *Artedius lateralis* and *Oligocottus maculosus*, and subtidal *Myoxycephalus polyacanthocephalus* and *Leptocottus armatus* (Figure 1, Table S1). Subtidal species represent outgroups to intertidal species, as well as the primary family Psychrolutidae. Phylogenetic designations follow Smith and Busby [35]. These species were chosen because they represent four different genera, show variation in contrasting streamlined or gibbose body profiles within each habitat, and individuals could be collected at similar body sizes from adjacent, shoreline habits. Although subtidal fish used in this study were juveniles, their presence within a short distance of intertidal habitats suggests they would have a higher likelihood of encountering similar flow demands as intertidal species, including the potential to get trapped in tidepools. Therefore, if there are differences compared to the adults of subtidal species, we expect that juveniles would show more exaggerated flow-related traits.

Two individuals of each species were collected on San Juan Island, WA during the summer of 2022. Following behavioral studies not included here, fish were euthanized via immersion in tricaine methanesulfonate (MS-222), fixed in 10% formalin for 48 hours, and stored in 70% ethanol. One additional individual per species was provided by the University of Washington Burke Museum ichthyology collection (Table S1). These individuals were examined to broaden the temporal and spatial representation of samples. Museum specimens were selected to represent recent collections from sites within the Salish Sea and Puget Sound but at a distance from Friday Harbor, and at similar body sizes to the specimens collected in 2022. Unfortunately, epidermal quality of specimens obtained from the Burke Museum was poor (Figure S1), rendering data collection difficult. Data from museum specimens were excluded from further analysis, though they showed qualitative similarities to freshly collected specimens.

Scanning Electron Microscopy

Right pectoral and pelvic fins were dissected at the base, dehydrated in 100% acetone, critical point dried, and sputter coated with a 10-12 nm gold coating. The left pectoral fin was used for specimen Alat07 because the right side was previously dissected. Samples were mounted to facilitate imaging the ventral surface of fin rays in a perpendicular plane to the electron beam. Imaging was performed with a Hitachi S3000N or Thermo Scientific Scios 2 DualBeam scanning electron microscope (SEM). Images (Hitachi: 1280 x 900 pixels; Scios 1536 x 1094 pixels) were taken at 1500X at the approximate midpoint along the free margin of each of the 5 ventral-most regionalized fin pectoral fin rays and the first pelvic fin ray. Some fin rays required multiple images because of the three-dimensional complexity of fin ray shape after critical point drying and the limits of tilting the stage on the SEM. For *Oligocottus maculosus*, each fin ray was imaged along multiple regions of the free margin to explore morphological variability within a single species and individual. This species was targeted because it is the most constrained to an intertidal habitat and will be the focus of follow-up work. Additional images were taken at lower magnifications to verify orientation and document other nearby features, but these were not used for analysis.

93 Following imaging, FIJI (https://fiji.sc/) was used to divide each picture into
94 quadrants of 1000 μm each (Figure S2) to quantify cell density and protruded area from
95 at least 4 quadrants per fin ray. Cell density was calculated by counting the total number
96 of cells per quadrant, including partial cells along the edges. Protruded area was
97 determined by tracing the outline of the protruded portion (containing the fibrils) of 3
98 cells per quadrant. Cells for protruded area measurement were selected based on the
99 edges of the fibrillar region being visible, perpendicular to the camera view, and to
100 encompass the shape and size variation of protrusions within the quadrant. Density and
101 area measures were averaged across quadrants for each image and then across
102 images for each fin ray, except for *O. maculosus*. For this species, measures were only
103 averaged across quadrants as each image corresponded to different regions along the
104 proximodistal axis of the fin ray.
105 To explore the morphometrics of the fibrils covering the surface of cells as well
106 as the width of the channels between the protruded area of cells, we selected the single
107 highest quality image of the pectoral fin ray surface for each individual at 1500x
108 magnification and made estimates of fibril density, diameter, and length, as well as
109 channel width (Figure S3). Pelvic fins were excluded for these measurements because
110 of lower image quality. Three protruded areas (or cells) per image per specimen and
111 three fibrils per cell were selected for measurement. Fibril density was estimated by
112 counting the number of fibrils within a specified area (usually protruded area, except for
113 *L. armatus* where cells were indistinguishable). Fibril diameter was estimated by
114 measuring the diameter of the fibril's tip, while fibril length was estimated by measuring
115 the length of a line from the base to the tip of the fibril. Repeat measures across cells or
116 fibrils were averaged per individual and then an average value ± standard error
117 calculated per species.
118
119 **Results**
120 All four sculpin species examined displayed a ventral fin ray pad with a
121 microtextured epidermal surface composed of fibrillar projections along the free margin
122 of pectoral and pelvic fin rays (Figure 1). In some species, the fibrillar projections were
123 arranged in groups separated by distinct channels, creating a more complex,

hierarchically textured surface. In contrast, the typical reticulated epidermal surface was present in areas located on the fin web, at the base of the fins, on the dorsal region of pectoral fins, and on non-primary pelvic fin rays (Figure 3). The two intertidal species (*A. lateralis* and *O. maculosus*) displayed the most prominent and easily distinguishable microtextured cells separated by channels up to approximately 2 μm in width (Table 1). Subtidal *M. polyacanthacephalus* had intermediate features that were distinguishable in some images and on some fin rays but were usually less prominent compared to the intertidal species, and channels were narrower compared to the intertidal species (Table 1). Subtidal *L. armatus* had the least well-defined cells, rendering cell morphometrics (cell density, protruded area, and channel width) unable to be measured.

Overall, there was substantive variation in cell morphometrics within and across species. Larger individuals generally had higher cell densities on their pectoral fins and lower densities on their pelvic fins (Figure 2). However, cell density was largely similar across species relative to the variation (Table 1, Figure 2). On average, *M. polyacanthocephalus* had the greatest protruded areas, especially on pectoral fins, and *O. maculosus* had the lowest protruded areas (Table 1, Figure 2). No obvious patterns were observed when comparing across fin rays relative to the variation.

Fibrillar projections appeared to vary in density among species between approximately 1-2 fibrils per μm^2 (Table 1). Subtidal species tended to be intermediate to intertidal species, which possessed the greatest (*O. maculosus*) and least (*A. lateralis*) dense fibrils. In contrast, *A. lateralis* had the greatest fibril length of just over 2 μm but also had the most variable fibril length compared to the other species. The smallest fibril length was just over 1 μm in *M. polyacanthocephalus*. Fibril diameter was, on average, similar across all species (Table 1).

Discussion

Sculpins are often used as a model of station-holding morphology, behavior, and function in benthic fishes without discrete attachment devices [8,11,20,26,34,36]. We documented microscopic projections on the ventral paired fin pads of marine sculpins that support novel mechanisms for benthic station-holding in this group of fishes that have not been previously considered or observed, despite an examination of fin ray

155 histology in benthic fishes that included a freshwater sculpin species [28]. We
156 hypothesize that these features are homologous to and elaborations of the typical
157 microridges observed on epidermal tissue [reviewed in 37] based on the presence of
158 morphological transitional states near the edges of the ventral fin pads (Figure 3).
159 Microridges are common features of vertebrate (and some invertebrate) epidermal
160 tissues, bearing anatomical and functional similarity to microvilli [37,38] but with
161 characteristically short heights rarely more than 1 μm [38,39]. Microridges are initially
162 formed as individual 'pegs' or 'puncta' that continuously elongate and divide laterally
163 through fission and fusion dynamics as a result of changes in membrane tension and
164 compression [37,40–43]. The resulting topography is highly plastic, shifting through the
165 life of the cell [38,40,42] and responding to factors such as cell division, epidermal
166 damage, hormones, environmental salinity, and even swimming activity level
167 [37,38,40,42,43]. Most authors suggest that their function is in mucous retention [citing
168 44]. Other non-mutually exclusive functions may include reinforcement of integument,
169 protection from abrasion, increasing cell surface area, serving as an F-actin reserve,
170 and facilitating signal transduction in response to external stimuli [37,40,43]. Given
171 these characteristics, it seems feasible that the fibrillar projections observed on sculpin
172 ventral fin pads may represent developmental modifications in the vertical and lateral
173 growth patterns of microridges. We suggest the descriptor 'filamentous microridges' for
174 these features, which was used to describe a similar epidermal texture on hillstream
175 fishes [45].However, this terminology is contingent upon the support of structural
176 homologies in future research.
177 Similar modified epidermal projections have been described on the fins and
178 mouthparts of river-dwelling Ostariophysan fishes such as loaches and algae
179 eaters, where they are termed 'unculi' [27,28]. The filamentous microridges we
180 observed in sculpins appear to be morphologically similar to the unculi on the ventral
181 surface of the pectoral and pelvic fins of *Paracrossocheilus* sp. and *Characidium* sp.
182 (Figure 3G) [27], though these features are considerably wider and longer than the
183 fibrils observed in sculpins, which have diameters at the submicrometer scale and
184 lengths of only a few micrometers. Further, in Ostariophysians, a single unculus adorns
185 each cell, while multiple fibrillar projections appear to emanate from a single cell in

186 sculpins. However, some unculi cluster together forming multicellular tubercles with
187 discrete channels separating the clusters, as observed on the rostral cap of
188 *Paracrossocheilus* sp. and *Garra gotyla* [27] (Figure 3D-E). These bear a striking
189 resemblance to the structures we observed in sculpins except that they are multicellular
190 clusters of unculiferous cells in these fishes and unicellular clusters of fibrillar
191 projections in sculpins. The structures that may bear the most resemblance to sculpins
192 are the unculi on the inner lip of *P. vittatus* (Figure 3F), which are unicellular and
193 approximately 2 μm tall, however, they appear in a broad plateau-like morphology. Reef
194 blennies also show microstructures grossly similar to sculpins, referred to simply as a
195 'cuticle' [22,28,46,47], but it is unclear how scale and architecture compare to sculpins.
196 Similar to the unculi of Ostariophysians, we hypothesize that the filamentous
197 microridges observed in sculpins enhance mechanical interactions with surfaces
198 through friction and/or adhesion, thereby contributing to station-holding performance.
199 These fibrils and their hierarchical organization in cells with discrete channels between
200 them are remarkably similar in their basic form to features deemed essential in
201 generating reversible attachment in the discrete attachment systems of other aquatic
202 organisms (Table 1). Discrete cells packed with fibrils are observed in both the adhesive
203 suction disc of clingfish [48–50], which live in similar environments as the species
204 studied here, and the adhesive toepads of tree and torrent frogs [reviewed in 43]. In
205 clingfish, raised, polygonal "papillae" are divided by narrow channels that line the edge
206 of the suction disc. Papillae are composed of bundles of hierarchical filaments that
207 branch into nanoscopic rods. [48,49,51]. The adhesive toepads of tree and torrent frogs
208 are also adorned with raised, polygonal epidermal cells separated by narrow channels
209 and are composed of nanopillars that also have discrete channels separating them [52].
210 The configuration of sculpin epidermal cells appears to be most similar to that of tree
211 and torrent frogs (though with smaller protruded areas and greater densities) with
212 filaments that are more similar to the nanoscopic rods observed in clingfish (Table 1).
213 The epidermal cells and fibrils in sculpins likely operate using similar principles
214 as observed or hypothesized in other taxa with discrete attachment systems. The fibrils
215 in sculpins may contact the surface directly and interdigitate with its asperities in a
216 process known as mechanical interlocking, similar to how the claws and setose

filaments of rheophilic insects, clingfish papillae, and tree frog epidermal cells may interact with surfaces [6,53,54]. Alternatively, or additionally, projections may increase friction with the substrate through adhesive interactions. Fish epidermis is typically coated in mucus, and perhaps especially so for mostly scaleless fishes such as sculpins [55]. Thus, increases in surface area by microscopic fibrils may enhance adhesion with the substrate via capillary or viscous forces, similar to what has been suggested in clingfish papillae or tree frog toepads [49,reviewed in 52]. Channels between epidermal cells may facilitate evacuation of excess mucus from the interface, decreasing the separation distance between the projections and substrate and increasing adhesive stress, similar to the mechanism hypothesized in the papillae of clingfish suction discs [49].

The microstructures observed in sculpins may also serve other or non-mutually exclusive functions. Ostariophysian unculi, for example, have been implicated in chemo- or mechanoreception, feeding, mechanical protection, or antifouling [27,29,56,57]. Sensory functions are unlikely in sculpin structures as sensory receptors such as tastebuds are larger, multicellular, or with distinct morphology unlike what is observed here [28,30,58,59]. Additionally, sensory receptors and unculiferous cells occur in non-overlapping locations in Ostariophysian fishes [28]. The presence of shorter, more robust unculi on the lips of fishes such as algae eaters (Figure 3F) suggests that mechanical abrasion may assist with feeding [27]. However, this is less likely to be an important function of fin-based epidermal microstructures, especially in suction-feeding fish such as sculpins [60–62]. Epidermal features may also provide mechanical protection from scrapes on rough substrates or from pathogens or other parasites. On some sculpin specimens, we observed what appeared to be sloughing of the outer epidermal layer (Figure 3H-I), revealing a similarly patterned layer below and suggesting that the outermost layer may be shed periodically. Skin sloughing has also been observed in the sculpin *Blepsias bilobus* in response to epibiotic growth [55] and in the unculiferous tissues of riverine Ostariophysian fishes, where the most well-developed unculi are present on the outermost layer following keratinization [28]. Epidermal microstructures are also reinforced in blennies [47,63], suggesting resistance to wear. In sculpins, the outer layer appears less developed and potentially worn (Figure

248 3I), suggesting that epidermal features may not be reinforced in sculpins. In either case,
249 microstructural wear likely reduces the potential of these structures to enhance surface
250 interactions. Thus, replacement through sloughing would likely be beneficial for both
251 increasing mechanical interactions but also protecting deeper tissue. So far, we have
252 not seen functional tests of these additional hypotheses in any fish known to have
253 epidermal microstructures.

254 We found considerable variation in the configuration and morphometrics of both
255 the epidermal cells and fibrillar projections in marine sculpins that suggest a greater
256 functional capacity in intertidal compared to subtidal species. Most notably, the
257 epidermal cells bearing fibrillar projections appear to be separated by discrete channels
258 that are widest in the environmentally more extreme intertidal species. Wider channels
259 in intertidal fishes may facilitate mucus evacuation for enhanced contact with the
260 substrate through mechanical or adhesive interactions relative to subtidal fishes [49],
261 which may be more critical in the chaotic intertidal zone. The intertidal species also
262 appear to have smaller protruded areas compared to the subtidal *M.*
263 *polyacanthocephalus*, likely a consequence of the wider channels but similar cell
264 densities. Preliminarily, fibrils appear to be longer in the intertidal species compared to
265 the subtidal species, which may increase surface area for adhesive interactions or
266 enable enhanced conformation with surface asperities through differential bending
267 properties [29,49]. These differences in the morphometrics of the fibrils should be
268 considered preliminary as specimen image quality and conditions were not ideal for
269 these measures. For example, projection densities are likely underestimated and
270 projection diameters are likely overestimated because fibrils may be coated in residual
271 mucus, rendering it difficult to know whether measures were of multiple fibrils clumped
272 together or fibrils with high morphological variability. Fibril lengths are likely
273 underestimated as it was difficult to orient the projections perfectly parallel to the
274 electron beam. Future work will need to optimize specimen preparation or employ
275 different imaging techniques (e.g., transmission electron microscopy) to better estimate
276 morphometrics of these structures. Additionally, a wider range of species and
277 individuals should be examined to further describe the potential specialization of
278 intertidal species. Although morphological differences between species may be

adaptations to the dynamic and extreme intertidal zone, invasion into intertidal habitats is a derived condition among marine sculpins that occurred once [2,35,64–66]. Thus, it is unclear whether these microscopic traits are associated with evolutionary history, environmental demands, or some combination of these factors. Nevertheless, our findings inspire new questions regarding how these features compliment other adaptations for benthic station-holding in sculpins, where traits such as small body size, gripping fin rays, and/or negative lift generation have been described as primary mechanisms [2,8,11,18,20,24,26,32,66–68].

Our observations suggest a new, convergent example of a morphological feature hypothesized to enhance friction and/or adhesion in aquatic vertebrates. This result represents a novel insight into the natural history of sculpins that is broadly applicable to further studies of organismal performance, attachment systems, and convergent evolution. This occurrence stretches the boundaries of our knowledge of benthic station-holding in fishes and highlights the need for inclusion of more taxa, including sculpins, in future research on friction enhancement and adhesion in biological systems.

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Data accessibility

All original and analysis images and data will be available on Dryad following publication. Alcohol preserved specimens and SEM preparations will be maintained in the Kane Lab at the University of Louisiana at Lafayette.

Ethics statement

All procedures involving the collection and housing of animals prior to preservation were approved by the Institutional Animal Care and Use Committee at the University of Washington (protocol #4238-18).

Artificial Intelligence (AI) declaration

Artificial intelligence was not used in the preparation of this manuscript.

References

1. Carlson RL, Lauder GV. 2011 Escaping the flow: boundary layer use by the darter Etheostoma tetrazonum (Percidae) during benthic station holding. *Journal of Experimental Biology* **214**, 1181–1193. (doi:10.1242/jeb.051938)

2. Buser TJ, Burns MD, López JA. 2017 Littorally adaptive? Testing the link between habitat, morphology, and reproduction in the intertidal sculpin subfamily Oligocottinae (Pisces: Cottoidea). *PeerJ* **5**, e3634. (doi:10.7717/peerj.3634)

3. Egan JP, Buser TJ, Burns MD, Simons AM, Hundt PJ. 2021 Patterns of Body Shape Diversity and Evolution in Intertidal and Subtidal Lineages of Combtooth Blennies (Blenniidae). *Integrative Organismal Biology* **3**. (doi:10.1093/iob/obab004)

4. Dodou D, Breedveld P, de Winter JCF, Dankelman J, van Leeuwen JL. 2011 Mechanisms of temporary adhesion in benthic animals. *Biological Reviews* **86**, 15–32. (doi:10.1111/j.1469-185X.2010.00132.x)

5. Stewart RJ, Ransom TC, Hlady V. 2011 Natural underwater adhesives. *Journal of Polymer Science Part B: Polymer Physics* **49**, 757–771. (doi:10.1002/polb.22256)

6. Ditsche P, Summers AP. 2014 Aquatic versus terrestrial attachment: water makes a difference. *Beilstein Journal of Nanotechnology* **5**, 2424–2439.

7. Delroisse J, Kang V, Gouveneaux A, Santos R, Flammang P. 2023 Convergent Evolution of Attachment Mechanisms in Aquatic Animals. In *Convergent Evolution: Animal Form and Function* (eds VL Bels, AP Russell), pp. 523–557. Cham: Springer International Publishing. (doi:10.1007/978-3-031-11441-0_16)

8. Webb PW. 1989 Station-holding by three species of benthic fishes. *Journal of Experimental Biology* **145**, 303–320.

9. Macdonnell AJ, Blake RW. 1990 Rheotaxis in Otocinclus sp. (Teleostei: Loricariidae). *Can. J. Zool.* **68**, 599–601. (doi:10.1139/z90-086)

10. Arnold GP, Webb PW, Holford BH. 1991 The role of the pectoral fins in station-holding of Atlantic salmon parr (Salmo salar L.). *J. Exp. Biol.* **156**, 625–629.

11. Webb PW, Gerstner CL, Minton ST. 1996 Station-holding by the mottled sculpin, Cottus bairdi (Teleostei: Cottidae), and other fishes. *Copeia* **1996**, 488–493.

12. Gerstner CL, Webb PW. 1998 The station-holding performance of the plaice Pleuronectes platessa on artificial substratum ripples. *Canadian Journal of Zoology* **76**, 260–268.

13. Wilga CD, Lauder GV. 2001 Functional morphology of the pectoral fins in bamboo sharks, Chiloscyllium plagiosum: benthic vs. pelagic station-holding. *Journal of Morphology* **249**, 195–209.

14. Parsons GR, Jeffrey Hoover J, Jack Killgore K. 2003 Effect of Pectoral Fin Ray Removal on Station-Holding Ability of Shovelnose Sturgeon. *North American Journal of Fisheries Management* **23**, 742–747. (doi:10.1577/m02-088)

15. Coombs S, Anderson E, Braun CB, Grosenbaugh M. 2007 The hydrodynamic footprint of a benthic, sedentary fish in unidirectional flow. *Journal of the Acoustical Society of America* **122**, 1227–1237. (doi:10.1121/1.2749455)

16. Gerstner CL. 2007 Effect of oral suction and other friction-enhancing behaviors on the station-holding performance of suckermouth catfish (Hypostomus spp.). *Canadian Journal of Zoology* **85**, 133–140. (doi:10.1139/Z06-199)

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

18. Hale ME, Galdston S, Arnold BW, Song C. 2022 The Water to Land Transition Submerged: Multifunctional Design of Pectoral Fins for Use in Swimming and in Association with Underwater Substrate. *Integrative and Comparative Biology* **62**, 908–921. (doi:10.1093/icb/icac061)

19. Fulton CF, Bellwood DB, Wainwright PW. 2001 The relationship between swimming ability and habitat use in wrasses (Labridae). *Marine Biology* **139**, 25–33. (doi:10.1007/s002270100565)

20. Taft NK, Taft BN. 2012 Functional implications of morphological specializations among the pectoral fin rays of the benthic longhorn sculpin. *The Journal of Experimental Biology* **215**, 2703–2710. (doi:10.1242/jeb.063958)

21. Binning SA, Ros AFH, Nusbaumer D, Roche DG. 2015 Physiological Plasticity to Water Flow Habitat in the Damselfish, Acanthochromis polyacanthus: Linking Phenotype to Performance. *PLOS ONE* **10**, e0121983. (doi:10.1371/journal.pone.0121983)

22. Brandstätter R, Misof B, Pazmandi C, Wagner GP. 1990 Micro-anatomy of the pectoral fin in blennies (Blenniini, Blennioidea, Teleostei). *Journal of Fish Biology* **37**, 729–743. (doi:10.1111/j.1095-8649.1990.tb02537.x)

23. Gosline WA. 1994 Function and structure in the paired fins of Scorpaeniform fishes. *Environ. Biol. Fishes* **40**, 219–226.

24. Taft NK, Lauder GV, Madden PGA. 2008 Functional regionalization of the pectoral fin of the benthic longhorn sculpin during station holding and swimming. *J. Zool.* **276**, 159–167. (doi:10.1111/j.1469-7998.2008.00472.x)

25. Taft NK. 2011 Functional implications of variation in pectoral fin ray morphology between fishes with different patterns of pectoral fin use. *Journal of Morphology* **272**, 1144–52. (doi:10.1002/jmor.10970)

26. Kane EA, Higham TE. 2012 Life in the flow lane: differences in pectoral fin morphology suggest transitions in station-holding demand across species of marine sculpin. *Zoology* **115**, 223–232. (doi:10.1016/j.zool.2012.03.002)

27. Roberts TR. 1982 Unculi (Horny Projections Arising from Single Cells), an Adaptive Feature of the Epidermis of Ostariophysan Fishes. *Zoologica Scripta* **11**, 55–76. (doi:10.1111/j.1463-6409.1982.tb00518.x)

28. Conway KW, Lujan NK, Lundberg JG, Mayden Richard L, Siegel DS. 2012 Microanatomy of the paired-fin pads of ostariophysan fishes (Teleostei: Ostariophysi). *Journal of Morphology* **273**, 1127–1149. (doi:10.1002/jmor.20049)

29. Chuang Y-C, Chang H-K, Liu G-L, Chen P-Y. 2017 Climbing upstream: Multi-scale structural characterization and underwater adhesion of the Pulin river loach (Sinogastromyzon puliensis). *Journal of the Mechanical Behavior of Biomedical Materials* **73**, 76–85. (doi:10.1016/j.jmbbm.2017.01.029)

30. Abd-Elhafeez H, Mokhtar D. 2014 Comparative Morphological Study of Lips and Associated Structures of Two Algal Grazer Fish. *Journal of Advanced Microscopy Research* **9**. (doi:10.1166/jamr.2014.1221)

31. White JL, Harvey BC. 1999 Habitat separation of prickly sculpin, Cottus asper, and coastrange sculpin, Cottus aleuticus, in the mainstem Smith River, northwestern California. *Copeia* , 371–375.

32. Kerfoot JR, Schaefer JF. 2006 Ecomorphology and habitat utilization of Cottus species. *Environ. Biol. Fishes* **76**, 1–13. (doi:10.1007/s10641-006-9000-0)

33. Buser TJ, Burns MD, López JA. 2017 Littorally adaptive? Testing the link between habitat, morphology, and reproduction in the intertidal sculpin subfamily Oligocottinae (Pisces: Cottoidea). *PeerJ* **5**, e3634. (doi:10.7717/peerj.3634)

34. Veillard MF, Ruppert JLW, Tierney K, Watkinson DA, Poesch M. 2017 Comparative swimming and station-holding ability of the threatened Rocky Mountain Sculpin (Cottus sp.) from four hydrologically distinct rivers. *Conservation Physiology* **5**, cox026. (doi:10.1093/conphys/cox026)

35. Smith WL, Busby MS. 2014 Phylogeny and taxonomy of sculpins, sandfishes, and snailfishes (Perciformes: Cottoidei) with comments on the phylogenetic significance of their early-life-history specializations. *Molecular Phylogenetics and Evolution* **79**, 332–352. (doi:10.1016/j.ympev.2014.06.028)

36. Kanter MJ, Coombs S. 2003 Rheotaxis and prey detection in uniform currents by Lake Michigan mottled sculpin (Cottus bairdi). *Journal of Experimental Biology* **206**, 59–70. (doi:10.1242/jeb.00056)

37. Depasquale JA. 2018 Actin Microridges. *The Anatomical Record* **301**, 2037–2050. (doi:10.1002/ar.23965)

38. Bereiter-Hahn J, Osborn M, Weber K, Vöth M. 1979 Filament organization and formation of microridges at the surface of fish epidermis. *Journal of Ultrastructure Research* **69**, 316–330. (doi:10.1016/S0022-5320(79)80050-7)

39. Sharma A, Anderson KI, Müller DJ. 2005 Actin microridges characterized by laser scanning confocal and atomic force microscopy. *FEBS Letters* **579**, 2001–2008. (doi:10.1016/j.febslet.2005.02.049)

40. DePasquale JA. 2018 Microridges in Cyprinus carpio scale epidermis. *Acta Zoologica* **99**, 158–168. (doi:10.1111/azo.12201)

41. Lu TQ, van Loon AP, Sagasti A. 2022 How to wrinkle a cell: Emerging mechanisms of microridge morphogenesis. *Current Opinion in Cell Biology* **76**, 102088. (doi:10.1016/j.ceb.2022.102088)

42. Lam P, Mangos S, Green JM, Reiser J, Huttenlocher A. 2015 In Vivo Imaging and Characterization of Actin Microridges. *PLOS ONE* **10**, e0115639. (doi:10.1371/journal.pone.0115639)

43. van Loon AP, Erofeev IS, Maryshev IV, Goryachev AB, Sagasti A. 2020 Cortical contraction drives the 3D patterning of epithelial cell surfaces. *Journal of Cell Biology* **219**, e201904144. (doi:10.1083/jcb.201904144)

44. Sperry DG, Wassersug RJ. 1976 A proposed function for microridges on epithelial cells. *The Anatomical Record* **185**, 253–257.

45. Joshi SH, Prieto-Marquez A, Parker WC. 2011 A landmark-free method for quantifying biological shape variation. *Biological Journal of the Linnean Society* **104**, 217–233.

46. Misof BY, Wagner GP. 1992 Regeneration in Salaria pavo (Blenniidae, Teleostei). *Anatomy and Embryology* **186**, 153–165. (doi:10.1007/BF00174953)

47. Wagner GP, Lo J, Laine R, Almeder M. 1993 Chitin in the epidermal cuticle of a vertebrate (Paralipophrys trigloides, Blenniidae, Teleostei). *Experientia* **49**, 317–319. (doi:10.1007/BF01923410)

48. Wainwright DK, Kleinteich T, Kleinteich A, Gorb SN, Summers AP. 2013 Stick tight: suction adhesion on irregular surfaces in the northern clingfish. *Biology Letters* **9**, 20130234. (doi:10.1098/rsbl.2013.0234)

49. Sandoval JA, Sommers J, Peddireddy KR, Robertson-Anderson RM, Tolley MT, Deheyn DD. 2020 Toward Bioinspired Wet Adhesives: Lessons from Assessing Surface Structures of the Suction Disc of Intertidal Clingfish. *ACS Appl. Mater. Interfaces* **12**, 45460–45475. (doi:10.1021/acsami.0c10749)

50. Sandoval JA, Jadhav S, Quan H, Deheyn DD, Tolley MT. 2019 Reversible adhesion to rough surfaces both in and out of water, inspired by the clingfish suction disc. *Bioinspiration & Biomimetics* **14**, 066016. (doi:10.1088/1748-3190/ab47d1)

51. Huie JM, Wainwright DK, Summers AP, Cohen KE. 2022 Sticky, stickier and stickiest – a comparison of adhesive performance in clingfish, lumpsuckers and snailfish. *Journal of Experimental Biology* **225**, jeb244821. (doi:10.1242/jeb.244821)

52. Langowski JKA, Dodou D, Kamperman M, van Leeuwen JL. 2018 Tree frog attachment: mechanisms, challenges, and perspectives. *Frontiers in Zoology* **15**, 32. (doi:10.1186/s12983-018-0273-x)

53. Ditsche-Kuru P, Koop JHE, Gorb SN. 2010 Underwater attachment in current: the role of setose attachment structures on the gills of the mayfly larvae Epeorus assimilis (Ephemeroptera, Heptageniidae). *Journal of Experimental Biology* **213**, 1950–1959. (doi:10.1242/jeb.037218)

54. Gorb SN. 2008 Biological attachment devices: exploring nature's diversity for biomimetics. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **366**, 1557–1574. (doi:10.1098/rsta.2007.2172)

55. Millstein J. 1998 Observations of Skin Sloughing in the Crested Sculpin (Blepsias bilobus). *Copeia* **1998**, 743–745. (doi:10.2307/1447807)

56. Das D, Nag TC. 2004 Adhesion by Paired Pectoral and Pelvic Fins in a Mountain-Stream Catfish, Pseudocheneis sulcatus (Sisoridae). *Environmental Biology of Fishes* **71**, 1–5. (doi:10.1023/B:EBFI.0000043148.23925.a7)

57. Das D, Nag TC. 2005 Structure of adhesive organ of the mountain-stream catfish, Pseudocheneis sulcatus (Teleostei: Sisoridae). *Acta Zoologica* **86**, 231–237. (doi:10.1111/j.1463-6395.2005.00202.x)

58. Ytteborg E, Hansen Ø, Høst V, Afanasyev S, Vieweg I, Nahrgang J, Krasnov A. 2020 Morphology, Transcriptomics and In Vitro Model of Skin from Polar Cod (Boreogadus Saida) and Atlantic Cod (Gadus Morhua). *Fishes* **5**, 34. (doi:10.3390/fishes5040034)

59. Cordero H, Ceballos-Francisco D, Cuesta A, Esteban MÁ. 2017 Dorso-ventral skin characterization of the farmed fish gilthead seabream (Sparus aurata). *PLOS ONE* **12**, e0180438. (doi:10.1371/journal.pone.0180438)

60. Norton SF. 1991 Capture success and diet of cottid fishes - the role of predator morphology and attack kinematics. *Ecology* **72**, 1807–1819.

61. Norton SF. 1995 A functional approach to ecomorphological patterns of feeding in cottid fishes. *Environmental Biology of Fishes* **44**, 61–78.

62. Kane EA, Higham TE. 2011 The integration of locomotion and prey capture in divergent cottid fishes: functional disparity despite morphological similarity. *Journal of Experimental Biology* **214**, 1092–1099. (doi:10.1242/jeb.052068)

63. Wagner GP. 1994 Evolution and multi-functionality of the chitin system. In *Molecular Ecology and Evolution: Approaches and Applications* (eds B Schierwater, B Streit, GP Wagner, R DeSalle), pp. 559–577. Basel: Birkhäuser Basel. (doi:10.1007/978-3-0348-7527-1_33)

64. Ramon ML, Knope ML. 2008 Molecular support for marine sculpin (Cottidae; Oligocottinae) diversification during the transition from the subtidal to intertidal habitat in the Northeastern Pacific Ocean. *Mol. Phylogenet. Evol.* **46**, 475–483. (doi:10.1016/j.ympev.2008.04.002)

65. Mandic M, Todgham AE, Richards JG. 2009 Mechanisms and evolution of hypoxia tolerance in fish. *Proc. R. Soc. B-Biol. Sci.* **276**, 735–744. (doi:10.1098/rspb.2008.1235)

66. Knope ML, Scales JA. 2013 Adaptive morphological shifts to novel habitats in marine sculpin fishes. *Journal of Evolutionary Biology* **26**, 472–482. (doi:10.1111/jeb.12088)

67. Bogdanov B. 2007 Variation of stone sculpin Paracottus knerii (Cottidae, Scorpaeniformes) of Baikal and waters of Baikal region. *Journal of Ichthyology* **47**, 162–174. (doi:10.1134/s003294520702004x)

68. Ingram T. 2011 Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society B-Biological Sciences* **278**, 613–618. (doi:10.1098/rspb.2010.1127)

69. Smith JM, Barnes WJonP, Downie JR, Ruxton GD. 2006 Structural correlates of increased adhesive efficiency with adult size in the toe pads of hylid tree frogs. *Journal of Comparative Physiology A* **192**, 1193–1204. (doi:10.1007/s00359-006-0151-4)

70. Endlein T, Barnes WJP, Jon P. 2015 Wet adhesion in tree and torrent frogs. *Encyclopedia of nanotechnology, Part* **2**, 1–20.

Table 1 Mean cell and fibril morphometrics across sculpin species examined, as well as estimates and ranges for comparative outgroups. Data are reported as means ± standard error.

Species	**Cell Density (mm^{-2})**	**Protruded Area (μm^2)**	**Fibril Density (μm^{-2})**	**Fibril Length (μm)**	**Fibril Diameter (μm)**	**Channel Width (μm)**
Leptocottus armatus	-	-	1.65 ± 0.11	1.31 ± 0.07	0.58 ± 0.04	-
Myoxocephalus polyacanthocephalus	40,340 ± 868	27.01 ± 1.58	1.49 ± 0.12	1.03 ± 0.21	0.57 ± 0.01	0.71 ± 0.0.03
Oligocottus maculosus	43,121 ± 2,100	16.16 ± 0.74	1.88 ± 0.003	1.54 ± 0.20	0.49 ± 0.05	1.97 ± 0.16
Artedius lateralis	40,122 ± 2,003	21.52 ± 1.37	1.20 ± 0.03	2.13 ± 1.05	0.65 ± 0.007	1.59 ± 0.40
Clingfish [48,49,51]	2,250	10,000	-	3 – 15	0.2 – 1.5	8
Tree frogs [52,69,70]	10,000	100	-	200 – 300	300 – 500	1 – 2

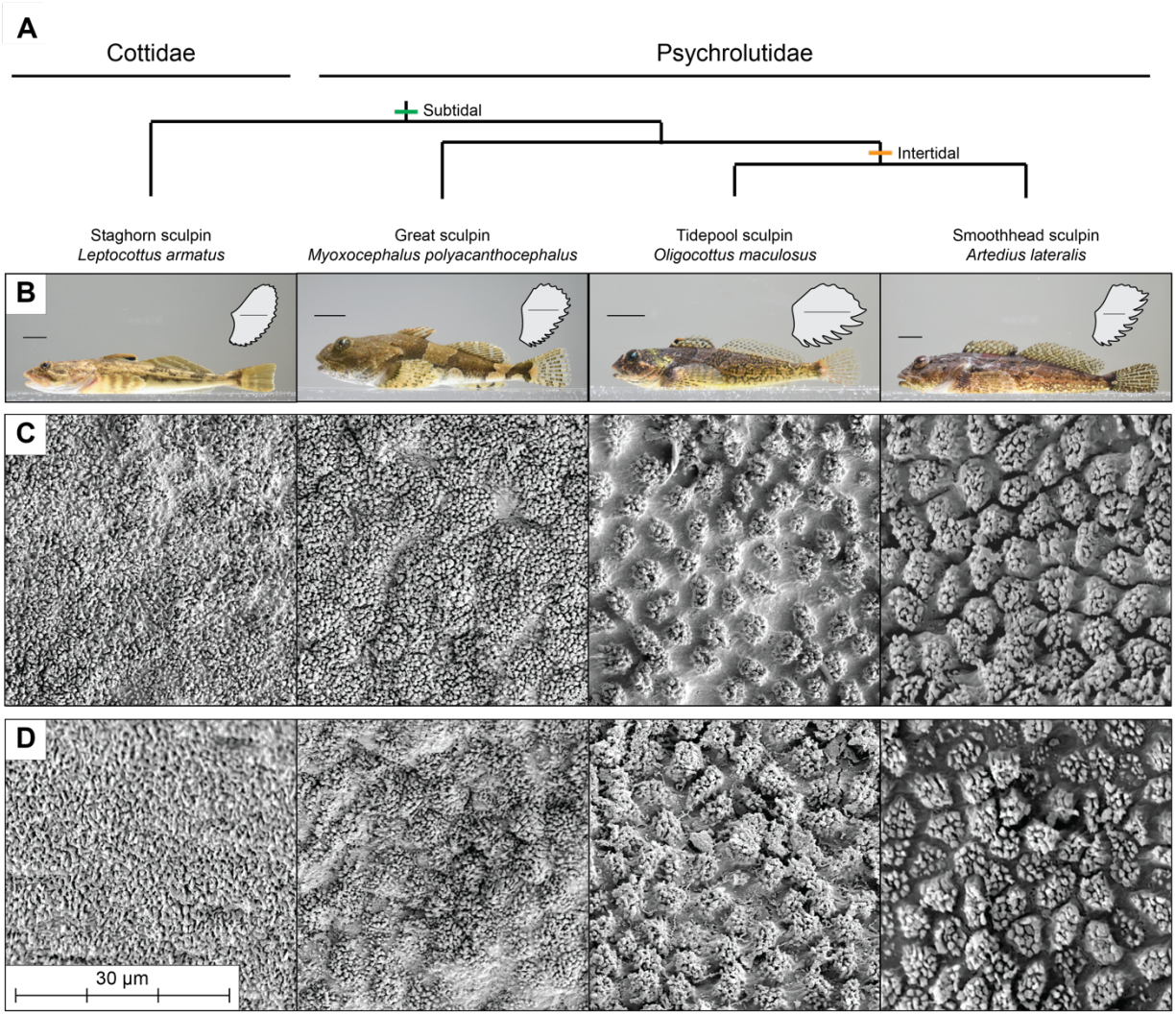

Figure 1 Overview of study species and results. A) Phylogenetic relationships of study species following Smith and Busby [35]. B) Lateral profile images of representative species from subtidal (green) and intertidal (orange) habitats. Images show fish collected in Summer 2022 but are not necessarily the individuals used in this work. Inset shows a tracing of a mounted pectoral fin of the individual photographed. Tracings are scaled to the same height. Scale bars are 1 cm. C-D) Representative scanning electron microscopy (SEM) images of a C) pectoral and D) pelvic fin rays of each species. Epidermal cells adorned with microscopic, fibrillar projections are present on all species. Channels separating protruded areas of epidermal cells are most defined in the two intertidal species.

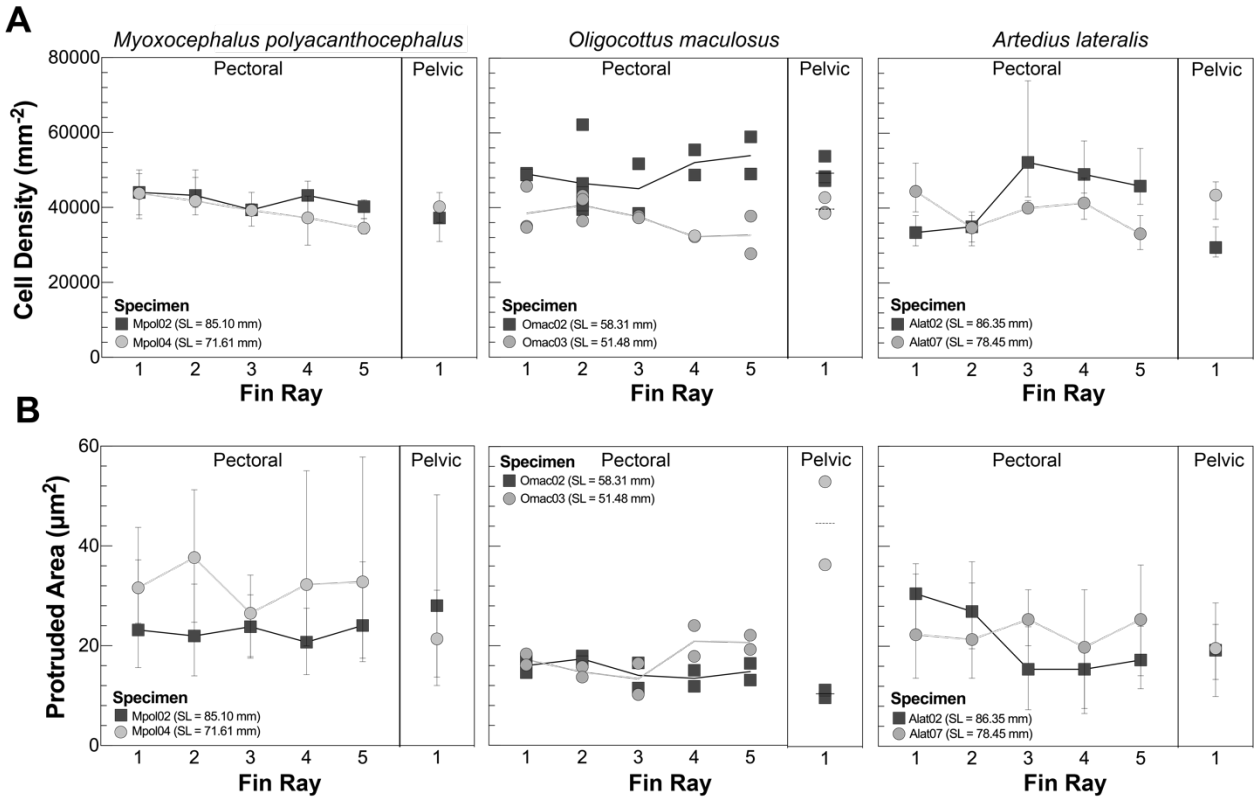

Figure 2 A) Cell density and B) protruded area measures across species, fins, and fin rays. Pectoral fin rays are ordered starting from the ventral-most ray. Density and area measures for *M. polyacanthocephalus* and *A. lateralis* are means calculated across all measured quadrants and images. Error bars represent the range of repeat measurements made per individual. For *O. maculosus*, each data point indicates the mean cell density or protruded area across measured quadrants taken at different locations along each fin ray per individual. All three species show similar cell densities. Protruded areas are greatest in subtidal *M. polyacanthocephalus*, lowest in *O. maculosus*, and intermediate in *A. lateralis*.

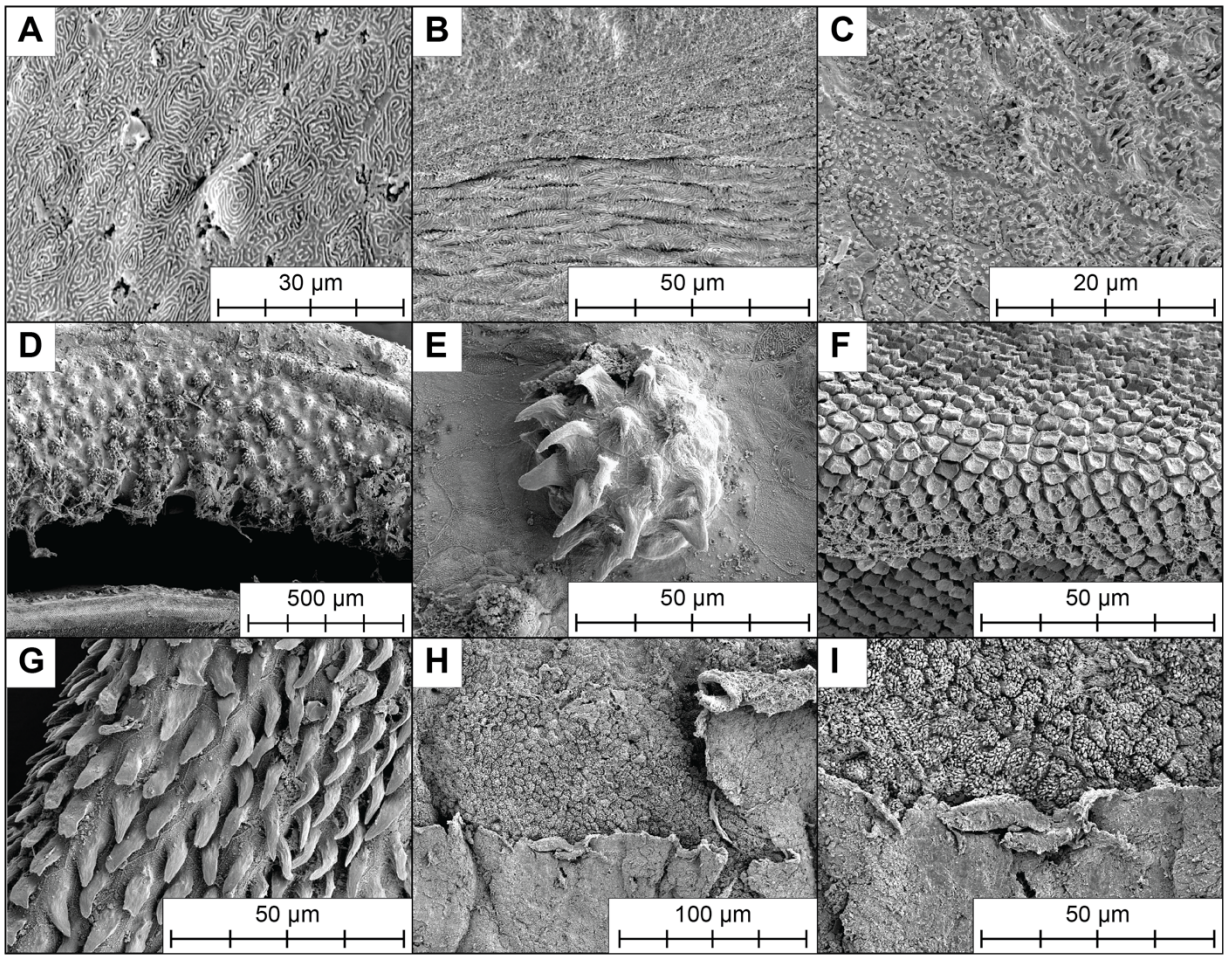

Figure 3 Representative scanning electron microscopy (SEM) images demonstrating additional observations that were not analyzed. A) Typical epidermis from the dorsal region of the pectoral fin of *A. lateralis*. B-C) The morphological transitional zone between typical epidermis near the pectoral fin webbing and modified epidermis on the edge of the ventral fin pad of (B) *O.maculosus* and (C) *A. lateralis.* D-G) Comparative images of the Ostariophysian *Paracrossochilus vittatus*: (E) multicellular unculiferous tubercle on the rostral cap, (F) hexagonal unculi on the inner lip, and (G) typical elongate unculi on the anterior pelvic fin ray. The specimen was a fresh preparation of a fish acquired in the aquarium trade following the methods described for sculpins. H-I) An example of skin sloughing observed in *M. polyacanthocephalus.*