

# Evolutionary optimization of an anatomical suction cup: Lip collagen content and its correlation with flow and substrate in Neotropical suckermouth catfishes (Loricarioidei)

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## Abstract

In riverine ecosystems, downstream drag caused by fast-flowing water poses a significant challenge to rheophilic organisms. In neotropical rivers, many members of a diverse radiation of suckermouth catfishes (Loricarioidei) resist drag in part by using modified lips that form an oral suction cup composed of thick flesh. Histological composition and morphology of this cup are interspecifically highly variable. Through an examination of 23 loricarioid species, we determined that the tissue most responsible for lip fleshiness is collagen. We hypothesized that lip collagen content is interspecifically correlated with substrate and flow so that fishes living on rocky substrates in high-flow environments have the largest, most collagenous lips. By mapping the amount and distribution of lip collagen onto a phylogeny and conducting ANOVA tests, we found support for this hypothesis. Moreover, these traits evolved multiple times in correlation with substrate and flow, suggesting they are an effective means for improving suction-based attachment. We hypothesize that collagen functions to reinforce oral suction cups, reducing the likelihood of slipping, buckling, and failure under high-flow, high-drag conditions. Macroevolutionary patterns among loricarioid catfishes suggest that for maximum performance, biomimetic suction cups should vary in material density according to drag and substrate requirements.

## KEYWORDS

biomimetics, comparative methods, histology, Loricariidae, morphology, phylogeny

## 1 | INTRODUCTION

Unidirectional downstream flow and consequent drag are ubiquitous features of rivers and streams that pose significant challenges to rheophilic organisms. Yet, despite the threat of displacement, high-flow habitats offer potential benefits to flow-specialized organisms, such as increased foraging efficiency and reduced predation pressure (see review by Lujan & Conway, 2015). Small, highly depressed organisms can largely escape the effects of flow-induced drag by occupying a thin boundary layer of low to zero flow that occurs close to substrate surfaces (Hoover & Ackerman, 2004). Similarly, many

specialized rapids-dwelling fishes escape the most intense flows by living in or among river substrates (Roberts & Stewart, 1976). In a landmark study of fishes specialized for life in the high-energy rapids of the lower Congo River, Roberts and Stewart (1976) grouped these flow-avoiding species together and compared them with the strongly rheophilic or current loving species, many of which had morphological specializations to counteract the current and resist dislodgement (Roberts & Stewart, 1976).

To overcome drag and maintain position within high-flow habitat patches, a broad geographic and phylogenetic diversity of rheophilic fishes have evolved similar sets of behavioral and morphological

specializations (Lujan & Conway, 2015). Some of these specializations improve swimming performance and endurance, such as the streamlined body and narrow caudal peduncle of the Bonytail Chub *Gila elegans*, which helps this species swim continuously in rapids (Moran, Ferry, & Gibb, 2016). Instead of escaping or actively overcoming high flow, other rheophilic fishes have developed more passive morphological and behavioral adaptations to engage with, and either attach to, or increase friction with substrate surfaces exposed to flow. Many rheophilic fishes such as Atlantic Salmon *Salmo salar* (Witten & Hall, 2002), parr, darters (*Etheostoma* sp.; Carlson & Lauder, 2010; Carlson & Lauder, 2011), and bamboo sharks use their bodies and/or pectoral fins as hydrofoils to produce negative lift and downforce on the substrate. Moreover, a wide range of ostariophysan fishes increase the frictional effect of fin-mediated downforce with thickened paired-fin pads covered ventrally with rear-facing, unicellular keratinous projections known as unculi (Conway, Lujan, Lundberg, Mayden, & Siegel, 2012).

One of the most distinctive passive specializations for high-flow or turbulent environments is an anatomical suction cup that some fishes use to generate negative pressure to assist with surface attachment (Fulcher & Motta, 2006). Suction cups that have evolved in fishes fit three broad categories: Whole body, modified fins, and modified mouths. Due to their flexible, highly depressed bodies, hillstream loaches (Balitoridae) are able to use their entire body as a sucker (De Meyer & Geerinckx, 2014). Clingfishes (Gobiosocidae), lumpsuckers (Cyclopteridae), and gobies (Gobiidae) have independently derived pelvic-fin modifications that allow them to act as a suction cup (Blob et al., 2019; Blob, Rai, Julius, & Schoenfuss, 2006; Davenport & Thorsteinsson, 1990; Ditsche, Wainwright, & Summers, 2014; Maie, Schoenfuss, & Blob, 2012; Schoenfuss & Blob, 2003; Wainwright, Kleinteich, Kleinteich, Gorb, & Summers, 2013), whereas remoras have a highly modified spiny dorsal fin that serves the same function (Beckert, Flammang, & Nadler, 2015; Fulcher & Motta, 2006; Nadler et al., 2013). Cypriniform algae-eaters (Gyrinocheilidae), some gobies, and suckermouth catfishes in both Africa (Mochokidae) and South America (Astroblepidae, Loricariidae) have mouths that are modified to act as oral suckers, allowing them to adhere to surfaces, often while simultaneously feeding and respiring (De Meyer & Geerinckx, 2014; Geerinckx, Brunain, Herrel, Aerts, & Adriaens, 2007; Gerstner, 2007; Macdonnell, 1990; MacDonnell & Blake, 1990; Maie et al., 2012; Schoenfuss & Blob, 2003). Such anatomical suction cups not only help fishes resist displacement in flowing water, but also aid some species in terrestrial locomotion. For example, some species have been observed climbing vertical surfaces outside of the water column (e.g., *Astroblepus* spp., *Cordylancistrus* sp., and *Sicyopterus stimpsoni*; Carvajal-Quintero, Maldonado-Ocampo, & Urbano-Bonilla, 2015; Evermann & Kendall, 1905; Johnson, 1912; Schoenfuss & Blob, 2003).

All fish suction cups share two important features: A relatively rigid support system and a flexible edge to form a seal. Hillstream loaches (Balitoridae) use their skeleton as a rigid support for their whole-body suction cup and use their soft fin rays and ventral unculi

to form a tight seal with the substrate (De Meyer & Geerinckx, 2014). In the pelvic suckers of clingfishes, the pelvic girdle provides support while microvilli form a flexible, peripheral seal even on irregular surfaces (Ditsche et al., 2014; Wainwright et al., 2013). In the highly diverse clade of over 1,000 Neotropical suckermouth catfishes (Astroblepidae + Loricariidae), an oral suction cup is formed by enlarged fleshy lips that are covered ventrally with unculiferous papillae (Geerinckx, Brunain, et al., 2007; Geerinckx, De Poorter, & Adriaens, 2007), and are supported and manipulated by the jaws and maxillary barbels (Geerinckx, Brunain, et al., 2007). This study is motivated in part by the observation that lip morphology in Neotropical suckermouth catfishes (families Astroblepidae and Loricariidae) is interspecifically highly variable, and aspects of this variation appear to correlate with species' preferred flow regimes and substrates, hinting at still-unknown fine-scale aspects of lip and oral disk function. To date, the soft internal structure of astroblepid and loricariid lips has not been examined in detail. Geerinckx, De Poorter, and Adriaens (2007) identified one muscle in the bottom lip, the *musculus intermandibularis anterior pars labialis*, but the identity of the remaining soft tissues that comprise the lips are unknown. Due to the apparent absence of muscles from the upper lip of these fishes (Geerinckx, Huysentruyt, & Adriaens, 2009), and the ubiquity of collagen as a semi-rigid soft tissue in vertebrates, we hypothesize that collagen is the principal soft tissue contributing to loricarioid lip fleshiness.

We further hypothesize that lip fleshiness of Neotropical suckermouth catfishes varies in response to the flow regime of habitats where they prefer to live, with species living in faster flows having fleshier lips with more collagen. Thicker, more collagenous lips are likely to create more robust suction cups, reducing the likelihood of failure under high flow and associated high drag. We predict that astroblepid and loricariid lip fleshiness correlates with preferred substrate type, because substrate strongly impacts fish performance in station-holding (Blake, 2006; Carlson & Lauder, 2010; Carlson & Lauder, 2011; Gerstner, 2007; Macdonnell, 1990; Witten & Hall, 2002) and adhesion (Blob et al., 2006; Ditsche et al., 2014; Gerstner, 2007; Macdonnell, 1990; Wainwright et al., 2013). We hypothesize that lips are fleshier in suckermouth catfishes that preferentially occupy solid bedrock or boulder substrates, versus softer substrates such as gravel, sand, or mud. Thus, the goals of this study are to quantify tissue composition of the oral disk suction cup across a broad phylogenetic diversity of neotropical suckermouth catfish species, quantify variance in the oral distribution of these tissues, and test whether lip tissue composition, or fleshiness, is phylogenetically correlated with flow or substrate type.

## 2 | METHODS

### 2.1 | Histology

Fifty-three formalin-fixed, alcohol-preserved specimens were obtained from the Auburn University Museum of Natural History fish collection. These comprised an outgroup of two armored catfish

(Callichthyidae) and one pencil catfish species (Trichomycteridae) and an ingroup of one naked suckermouth catfish (Astroblepidae) and 19 suckermouth armored catfish species (Loricariidae; Table S1). All specimens were cleared and stained for bone with Alizarin Red (Sigma Chemical Co., St. Louis, MO) using the protocol by Taylor and Van Dyke (1985). The clearing and staining process causes collagen to stand out as a whitish tissue (NRB personal observations), so by staining bones red, collagen was better contrasted with and delimited from the skeleton (Figure 1). To confirm that whitish tissue in the lips and anterior portion of the head was collagen, we stained a specimen of *Chaetostoma*, which have particularly fleshy lips, with the Verhoeff–Van Gieson Elastin Stain kit (Polysciences, Inc., Warrington, PA) for elastin (black) and collagen (red). This preserved specimen originating from the aquarium trade and is part of the private specimen collection of NRB. As protocols for staining with Verhoeff–Van Gieson are available only for slides, we used trial and error to modify the staining protocol that came with the kit for whole specimens as follows: (a) transfer specimen to xylene for at least 1 day, (b) hydrate specimens in distilled water, (c) stain in Verhoeff's solution for 5 hr, allowing the tissue to become completely black, (d) rinse in tap water with three changes, (e) differentiate in 2% ferric chloride for 10 min, (f) stop differentiation with several changes of tap water, (g) wash specimen in tap water for 15 min, (h) treat with 5% sodium thiosulfate for 10 min, (i) wash in running tap water for 10 min, (j) counter stain in Van Gieson's solution for 20 min, (k) dehydrate through 95% ethanol, two changes of 100% ethanol, (l) clear in two changes of xylene for 1 hr each, and (m) transfer to ethanol and grade up to 70% for storage. It should be noted that through this method, the stained tissue is not stable for permanent storage, as the red counterstained collagen fades to dark purple after about 1 year of storage.

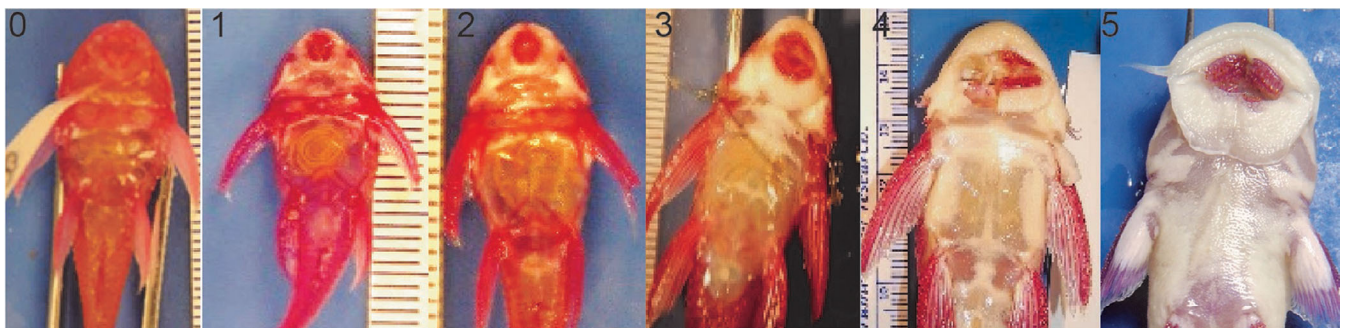
## 2.2 | Morphometrics and collagen measurements

After confirming that white tissue in the lips and anterior portion of the head was collagen (Figure 2), each specimen was photographed in dorsal and ventral view using a macro lens on a Nikon D50 digital



**FIGURE 2** Specimen of *Chaetostoma* sp. stained with Verhoeff–Van Gieson stain and counterstain. Collagen (red) is visible dorsally in the upper lip/anterior portion of the head, as well as throughout the skin. Elastin (black) is mostly visible in the dermal bones, but is largely absent in the upper lip/anterior portion of the head. This photo has been modified in Adobe Photoshop to remove the background and increase brightness, but has not been otherwise altered

camera for larger specimens, and a Leica MZ16 stereomicroscope for smaller specimens. The area of white collagenous tissue around the mouth/oral sucker was measured dorsally and ventrally using ImageJ software (Schindelin et al., 2012), as well as head width (HW), mouth width (MW) and length (ML), width at the widest part of the body (BW), top lip length (TLL), bottom lip length (BLL), and standard length (SL). Collagen areas were scaled to  $SL \times BW$  (CAS) and  $ML \times MW$  (CAM) to standardize measurements. The right mandible and lower lip had been dissected from some specimens for a previous project (Lujan & Armbruster, 2012), so collagen measurements of the intact left side of these specimens were doubled to compensate for this. For individuals with terminal rather than inferior mouths (e.g., the outgroups



**FIGURE 1** Oral collagen opacity scale. Examples of the cleared and stained catfishes are shown for each integer on the collagen opacity scale. Collagen appears as white tissue, whereas bone is stained red (0 = *Panaque bathyphilus*, AUM 45504; 1 = *Panaqolus albomaculatus*, AUM 45507; 2 = *Panaqolus gnomus*, AUM 45505; 3 = *Panaqolus nocturnus*, AUM 45563; 4 = *Chaetostoma stroumpoulos*, AUM 45663; 5 = *Astroblepus* sp., AUM 32834)

Callichthyidae and Trichomycteridae), morphometric data were taken using digital calipers. Collagen opacity was qualitatively recorded on a scale from 0 to 5 as a proxy for collagen density and thickness. The scoring system was as follows: 0, no visible collagen surrounding the mouth; 1, collagen apparent but translucent; 2, some collagen opaque; 3, most collagen opaque but some translucent; 4, almost all collagen completely opaque; 5, oral tissue completely white and opaque due to dense collagen.

## 2.3 | Flow and substrate classification

Flow and substrate classifications were assigned to each species independently of collagen observations by authors JWA and NKL based on extensive first-hand field observations made while collecting these fishes. Flow was broken into four categories: (a) low for species that live in pools, (b) medium for species that live in runs, (c) high for species that live in relatively rapid flow, generally riffles, rapids, or the swiftest areas in otherwise relatively slow streams, and (d) very high for species that live primarily in torrential montane habitats. Substrate was broken into three categories: (a) mud/sand, (b) logs and debris, and (c) rocks.

## 2.4 | Phylogenetic comparative methods and statistical analysis

A phylogeny was built in Mesquite ver. X based on the multi-locus study of Lujan et al. (2015). Because the outgroup taxa examined here were not included in Lujan et al. (2015), branch lengths were arbitrarily set to one. Phylogenetic independent contrast analyses were found to be robust regardless of branch length (Garland et al., 1992), and branch length likely would not have affected results at the scale of our analyses.

In order to plot character state changes for continuous characters, we used the function `contMap` in `phytools` (Revell, 2012). To plot discrete characters (flow and substrate), we used stochastic character mapping in `phytools` with the `simmap` function with 100 simulations and an equal rate (ER) model; colors were chosen to fit the character distributions of the continuous characters. Tests of phylogenetic

signal and model fit were performed in Geiger (Harmon, Weir, Brock, Glor, & Challenger, 2008; Pennell et al., 2014). Phylogenetic signal was tested with Blomberg's *K* and Pagel's Lambda using the `phylosig` function; values for *K* and lambda and *p* values are in Table 1. Blomberg's *K* provides a value from 0 to greater than 1 with 0 indicating no phylogenetic signal, 1 indicating Brownian motion, and greater than 1 indicating phylogenetic signal (Blomberg, Garland Jr, & Ives, 2003). Pagel's Lambda provides values from 0 to greater than 1 with 0 showing no phylogenetic signal, 1 indicating that morphological change is proportional to phylogenetic distance, and greater than 1 indicating that species are more morphologically similar than expected from phylogenetic distance alone (Pagel, 1999).

The following models were tested using the `fitcontinuous` function: Brownian motion, Pagel's Lambda, No Signal (performed by using the function `rescale` on the phylogeny, lambda as the model, and the value for lambda as 0, which creates a star phylogeny), Ornstein-Uhlenbeck, and Early Burst. Relative effectiveness of each model was assessed using the Akaike information criterion with correction for small sample sizes (AICc) in which values closer to zero indicate better fit.

Phylogenetic generalized least square (PGLS) ANOVAs were performed for scaled collagen measurements against flow and substrate using the `gls` function in R with the correlation being the expected covariance under a Brownian Motion model and maximum likelihood (`gls(Measurement~flow or substrate, correlation = corBrownian[phy = tree built above], data = all measurement table, method="ML")`). We ran a phylogenetic principal components analysis (pPCA) in `phytools` (Revell, 2009, 2012). A pPCA corrects data for non-independence due to phylogeny under different models of evolution. We ran the analysis under Brownian motion and Pagel's Lambda, and results were similar so only the Brownian motion model is presented. We additionally ran a `phylomorphospace` analysis (Sidlauskas, 2008) on the first two principal components in `phytools` using the `phylomorphospace` function, and the color-coded species to flow and substrate categories to visually identify the shape space occupied by clades and ecologies (Figure 3).

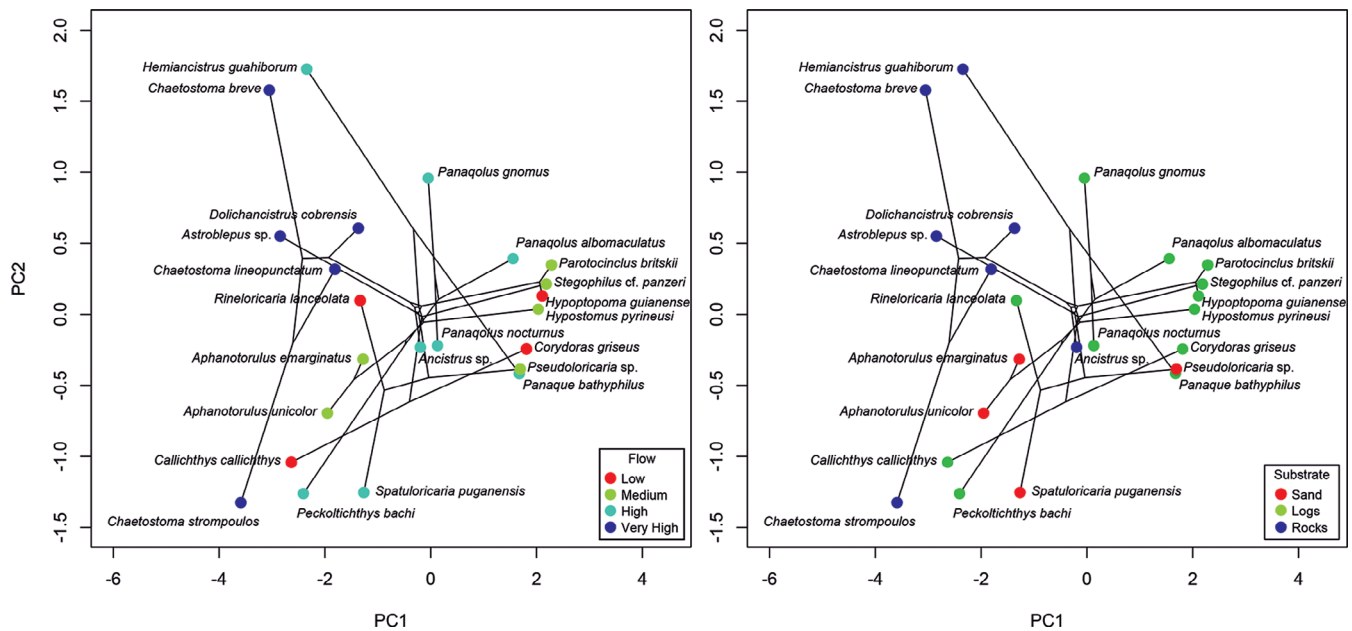
As the models did not fit well for flow, parameters in relation to flow were also analyzed assuming phylogenetic independence. Using the `aov()` function, analysis of variance (ANOVA) tests were performed in R (R Core Team, 2019) to compare morphometrics, collagen

**TABLE 1** Results of tests for phylogenetic signal and best fit models

| Character               | Brownian motion |      |      | Pagel's lambda |          |     | No signal<br>AICc | Ornstein-Uhlenbeck<br>AICc | Early burst<br>AICc |
|-------------------------|-----------------|------|------|----------------|----------|-----|-------------------|----------------------------|---------------------|
|                         | AICc            | K    | p    | AICc           | Lambda   | p   |                   |                            |                     |
| Scaled dorsal collagen  | −33.43          | 0.21 | .575 | −49.82         | 0.00007  | 1   | −52.48            | −49.82                     | −30.77              |
| Scaled ventral collagen | −85.65          | 0.39 | .060 | −90.68         | 0.677331 | .05 | −89.46            | −89.05                     | −82.99              |
| Scaled ventral to mouth | 124.01          | 0.16 | .864 | 102.43         | 0.00007  | 1   | 99.77             | 102.46                     | 126.68              |

Note: AICc is Akaike information criterion with correction for small sample sizes. Best fit models are those with an AICc closer to zero. Blomberg's *K* is provided for the Brownian motion model with a *K* near 1 indicating Brownian motion, values greater than 1 indicating phylogenetic signal, and values near zero indicating no phylogenetic signal. For Pagel's lambda, the lambda value is provided with a lambda of 1 indicating phylogenetic signal and of 0 indicating no signal. *p* values are provided for *K* and lambda.





**FIGURE 3** Results of the phylomorphospace analysis with species dots colored by flow (left) and substrate (right). Legends in the bottom right corner of each panel indicate colors corresponding to flow and substrate

opacity, and area of collagen scaled to SL. Post hoc Tukey honest significant differences (HSD) tests were performed using the TukeyHSD () function on data for which there were significant ANOVA results ( $p < .05$ ), to determine which groups were significantly different from each other.

### 3 | RESULTS

Verhoeff–Van Gieson stain indicated that collagen was the principal tissue composing the thick, fleshy lips of *Chaetostoma* sp., while elastin was macroscopically absent from the lips (Figure 2). Success of the staining procedure was confirmed by black staining of the ossified integumentary plates that cover the bodies of suckermouth armored catfishes (Figure 2), as fish bones are known to contain elastin and/or elastin-like proteins (Miyake, Vaglia, Witten, & Hall, 2001; Witten & Hall, 2002). Species examined in this study displayed considerable variation in their oral morphology and collagen distribution (Figure 1). While some species did not have collagen apparent ventrally, fewer had collagen dorsally. Most dorsal collagen was distributed anteriorly and connected to the anterior ventral collagen.

Tests for phylogenetic signal were generally not significant (Table 1). The only significant test was scaled ventral collagen under Pagel's Lambda; however, AICc tests showed that Pagel's Lambda was not the best fit model for the analysis. In general, AICc scores were highly positive or negative indicating poor fit of evolutionary models to the data; however, the only analysis where the “no signal” model had the best AICc score out of all tested models was ventral collagen area scaled to MW\*ML, suggesting that phylogeny does have some role in structuring the data.

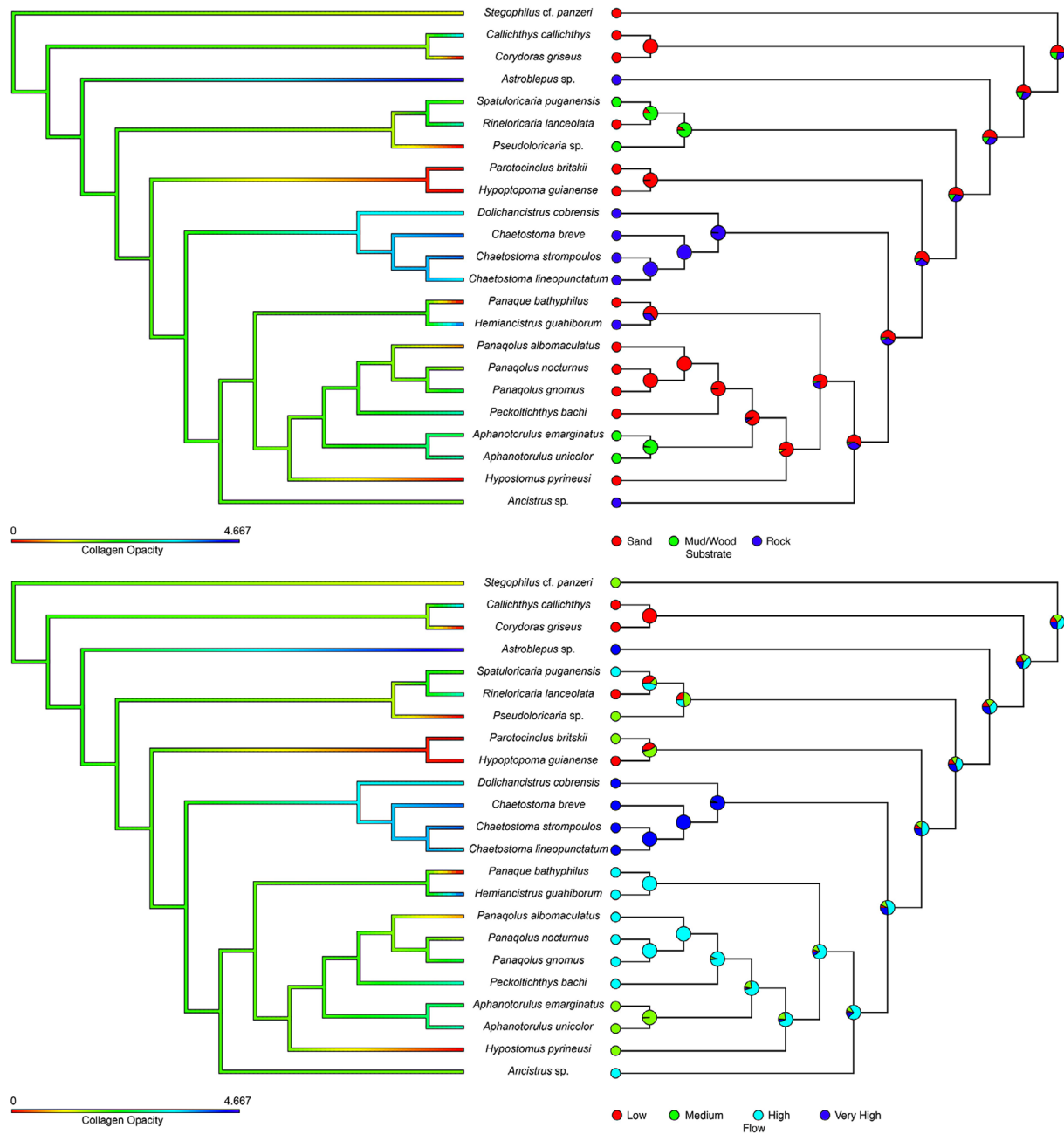
PGLS tests were not significant for flow, but were significant for the relationship between substrate and scaled dorsal and ventral collagen scaled to SL\*BW (Table 2). However, there was no significant effect of flow or substrate on CAM. Character change was visualized across the phylogeny for each of the scaled characters (scaled to TL\*BW; Figure 4, Figures S1 and S2) and one character with the greatest variance (Collagen Opacity) was compared to changes across flow and substrate (Figures 5 and 6). When viewed against each other (Figures 4 and 6, Figure S3), flow, collagen opacity, and our scaled measurements do appear to be correlated; however, small sample sizes likely contributed to nonsignificance. The fastest flow regime was assigned to only two clades: *Astroblepus* (Astroblepidae) and the *Chaetostoma/Dolichancistrus* clade (Loricariidae). Substrate, however, was significantly correlated with collagen, indicating that species occupying rocky substrates have more collagen than those found on wood or sand (Figure 5). Ignoring phylogeny, species from higher flow environments had significantly greater MW scaled to TL\*BW, ventral and dorsal CAS and collagen opacity (Table 3; Figure 5).

Results of the pPCA indicate that collagen opacity and dorsal collagen area have a strong positive effect on PC2, and collagen area,

**TABLE 2** Significance ( $p$ ) of phylogenetic generalized least squares ANOVAs for scaled collagen measurements versus flow and substrate

| Character                        | Flow   | Substrate |
|----------------------------------|--------|-----------|
| Scaled ventral collagen area     | 0.2850 | 0.0012*   |
| Scaled dorsal collagen area      | 0.2727 | 0.0474*   |
| Scaled ventral collagen to mouth | 0.1656 | 0.2950    |

Note: An asterisk (\*) denotes significance at the  $\alpha = .05$  level.



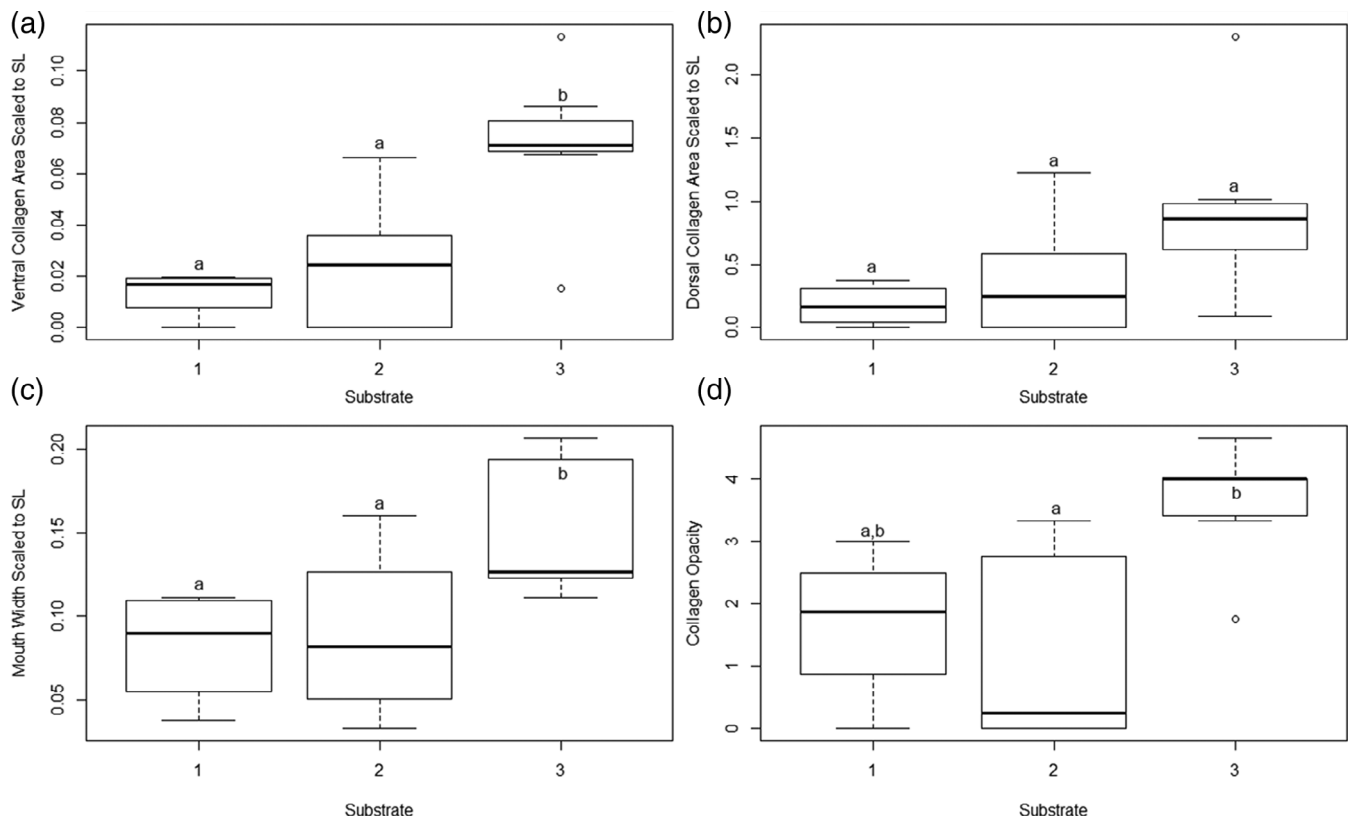
**FIGURE 4** Distribution of collagen opacity (left) across the phylogeny versus substrate (right, top) and flow (right, bottom). For the discrete characters on the right, state probability at nodes is indicated via pie charts. Colors correspond to Figure 3

MW, HW, and BW have strong negative effects (Figure 7). Effects of measurements were smaller in PC1 (which is more common in pPCA than in standard PCA's; Polly, Lawing, Fabre, & Goswami, 2013) with collagen opacity, collagen area, HW, MW, and BW all having strong negative effects and nothing having a positive effect (Figure 7). Species occupying higher flow and rocky habitats generally had higher negative PC1 scores and those living on sand had low PC2 scores (Figure 7). Although high, medium, and low flow and sandy and rocky habitats seem to be dispersed through the phylogeny, rocky habitats and especially very high flow habitats likely have some phylogenetic signal as all members of the *Chaetostoma*/*Dolichancistrus* clade have

these traits, whereas both traits are less common in other clades (Figure 3).

## 4 | DISCUSSION

Our comparative analyses significantly correlate collagen in the oral suction cup of a diverse subset of Neotropical suckermouth catfishes with substrate, but the results for flow were less clear and nonsignificant according to this analysis. Nevertheless, positive trends in the association between collagen and flow are apparent in the distribution



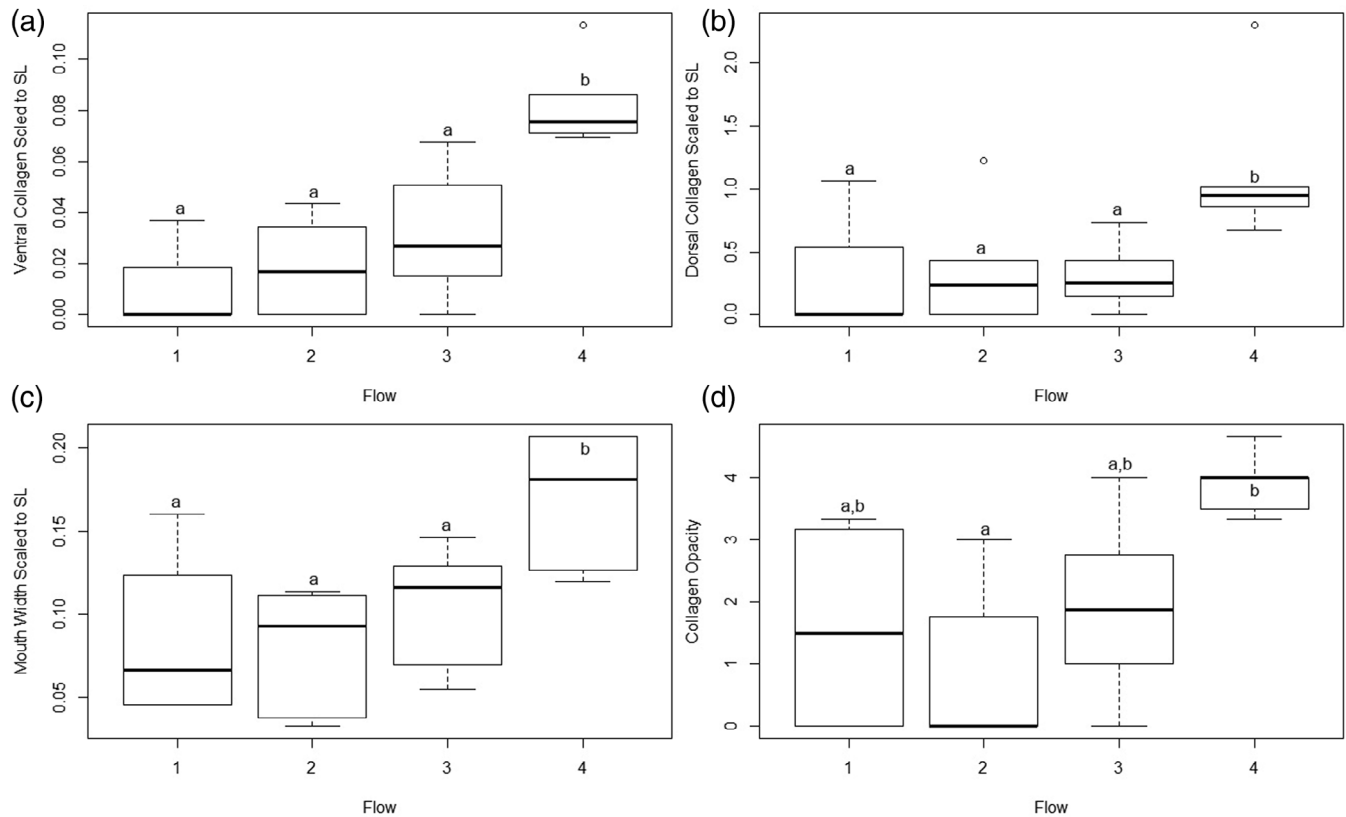
**FIGURE 5** Boxplots and post hoc Tukey results for substrate compared to collagen measurements. Collagen and mouth parameters for sandy (1) and woody (2) substrates are similar to each other, but significantly different than for rocky substrates (3). Lowercase letters on each boxplot represent Tukey results, with different letters corresponding to significantly different groups at the  $\alpha = .05$  level

of characters on the phylogeny (Figure 4), the phylomorphospace plot (Figure 7) and data that were not phylogenetically corrected (Table 1; Figure 6). The failure of phylogenetically corrected data to support this trend may be explained by our examination of only two lineages from the fastest flow category (i.e., *Astroblepus* and the *Chaetostoma/Dolichancistrus* clades). We speculate that if more fast-water lineages were included (e.g., Delturinae, Lithogeninae, *Lithoxancistrus*, *Pseudancistrus*) we would see greater phylogenetic significance. Moreover, we interpret the significant relationship between substrate and oral disk collagen as being at least partly driven by flow, since flow regulates the distribution of substrate sizes in streams, such that sand and wood accumulate in relatively lentic zones (Tett, Gallegos, Kelly, Hornberger, & Cosby, 1978). When collagen area measurements were scaled to mouth dimensions rather than body dimensions, though, there were no significant correlations with flow or substrate, whether phylogenetically corrected or not (Tables 2 and 3). This suggests that differences in mouth shapes between species effect the amount of oral collagen, and may affect station-holding and suction performance.

Fishes in low flow, sandy environments have relatively low station-holding demands as drag in such habitats is lower, and adherence via suction to loose particles difficult. Indeed, many Neotropical suckermouth catfishes that are specialized for such habitats (e.g., many Loricariinae; Covain & Fisch-Muller, 2007) behaviorally escape flows by burying themselves. Most of these species also have

oral disks that are highly fimbriate and specialized for detecting food in soft substrates rather than for forming an effective suction cup.

Higher flows cause fishes to experience greater drag (Blake, 2006). For fishes that station-hold via suction, water velocities that they can passively resist are those in which the suction cup does not slip or fail. Under extreme flows and drag, increased friction to prevent suction cup slippage can play an important role. Loricarioids likely achieve this by having larger lips, uncili covering the ventral surface of their oral disks (Ono, 1980), and papillae around the oral disk margin that help form a seal against surfaces similar to how marginal microvilli facilitate gobiosocid pelvic sucker adhesion (Ditsche et al., 2014; Wainwright et al., 2013). *Chaetostoma* spp., for example, have among the largest, thickest, most collagenous lips of any loricarioid for their size and live mostly in streams of the Andes Mountains, which are some of the highest flow environments in South America. The benefit of larger lips in *Chaetostoma* is supported by Blake (2006), who found this genus to have the highest slipping velocity of the five loricariid species he investigated. In addition to slippage, suction cup failure occurs when edges of the cup slide towards the center, eventually buckling inwards (Wainwright et al., 2013). Collagen is a tough, semi-rigid tissue that may help prevent the edges of oral suckers from sliding inward, so loricarioid species in higher flows likely need more of it to reinforce their oral suction cups, and reduce the probability of suction cup failure.



**FIGURE 6** Boxplots and post hoc Tukey results for flow compared to collagen measurements. Collagen area, collagen opacity, and mouth width generally increase with increasing flow (1 = pool, 2 = run, 3 = riffle, 4 = very high flow). Lowercase letters on each boxplot represent Tukey results, with different letters corresponding to significantly different groups at the  $\alpha = .05$  level

| Parameters                                       | F value | p value   | df |
|--|---------|-----------|----|
| Flow: Mouth width                                | 5.111   | .00924*   | 3  |
| Flow: Collagen opacity                           | 5.594   | .00636*   | 3  |
| Flow: Dorsal collagen scaled to body area        | 4.466   | .0155*    | 3  |
| Flow: Ventral collagen scaled to body area       | 12.49   | 9.63E-05* | 3  |
| Flow: Ventral collagen scaled to mouth area      | 0.731   | .546      | 3  |
| Substrate: Mouth width                           | 6.055   | .00879*   | 2  |
| Substrate: Collagen opacity                      | 8.542   | .00208*   | 2  |
| Substrate: Dorsal collagen scaled to body area   | 3.893   | .0373*    | 2  |
| Substrate: Central collagen scaled to body area  | 11.83   | .000406*  | 2  |
| Substrate: Ventral collagen scaled to mouth area | 0.650   | .553      | 2  |
| Substrate: Flow                                  | 11.16   | .000556*  | 2  |

Note: An asterisk (\*) denotes significance at the  $\alpha = .05$  level.

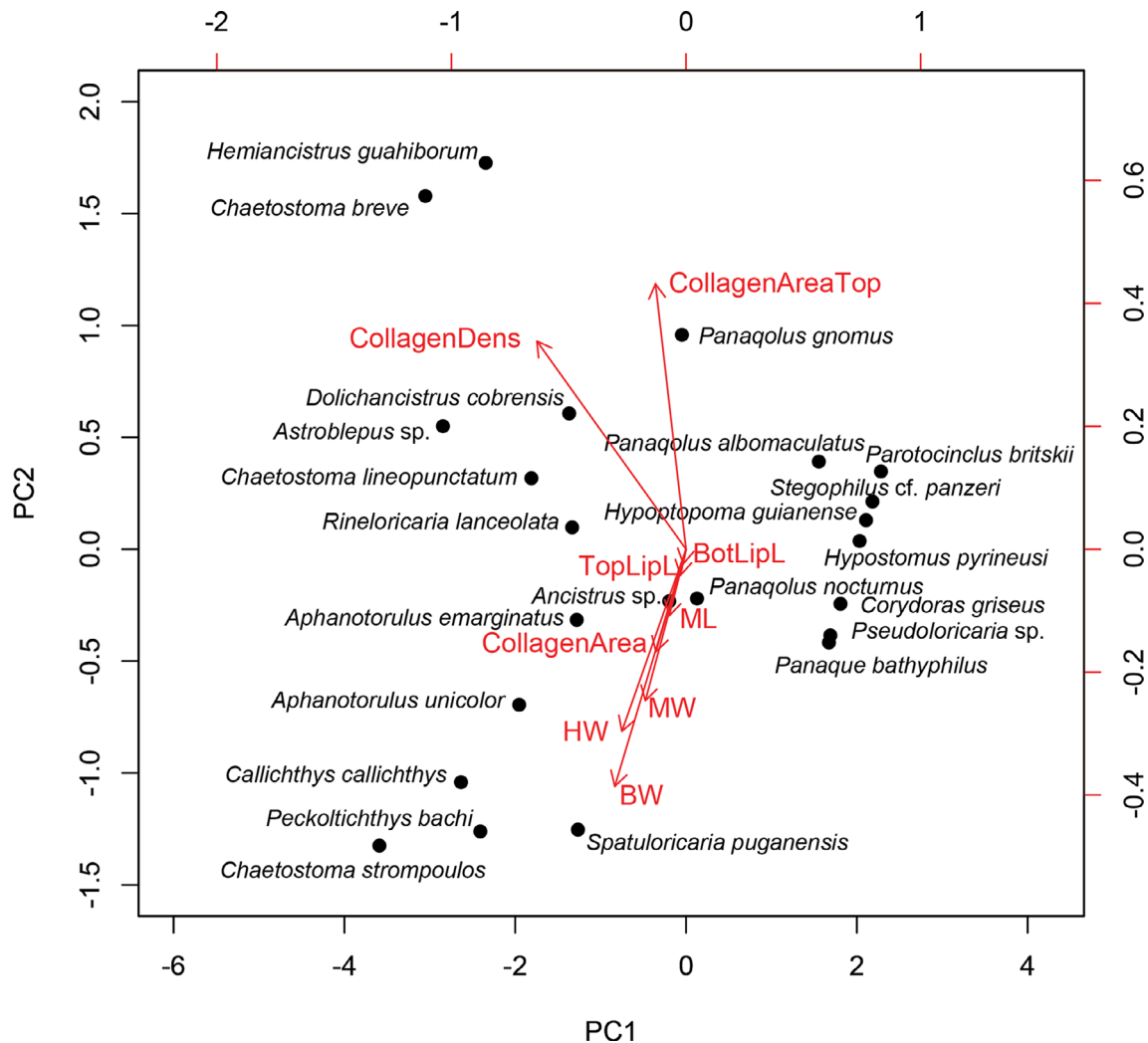
**TABLE 3** ANOVA results assuming phylogenetic independence

The taxon with the most oral-disk collagen in our study was *Astroblepus*, species of which often co-occur with *Chaetostoma*, or occupy even higher elevation and more torrential Andean stream habits than those of *Chaetostoma* (Schaefer & Arroyave, 2010). Some *Astroblepus* can even climb waterfalls by using their oral sucker in combination with a unique, highly mobile pelvic girdle (Evermann & Kendall, 1905; Johnson, 1912). Large, collagenous lips help *Astroblepus* meet their exceptional requirements for adhesion and

station-holding by increasing friction to prevent slippage under torrential drag and providing structural reinforcement to prevent their oral disk sucker from failing.

Our results indicate that loriciarioid catfishes have evolved large, collagenous lips multiple times (Figure 4) in correlation with high flow environments and rocky substrates, and that species of *Astroblepus* and *Chaetostoma* have the thickest, most collagenous lips, making them excellent models for future experimental research. Although it





**FIGURE 7** Results of the pPCA with component loadings in red. BLL, bottom lip length; BW, body width; collagen area bottom, ventral collagen area scaled to body area; collagen area top, dorsal collagen area scaled to body area; HW, head width; ML, mouth length; MW, mouth width; TLL, top lip length

seems clear that these specializations improve station-holding by delaying suction cup failure under high flow conditions, additional experiments are needed to better understand how loricariid oral disks interact with different substrates. Research has demonstrated that substrate surface features at a range of spatial scales can impact station-holding performance in other fishes, yet this still needs testing in loricarioids (Blake, 2006; Carlson & Lauder, 2010; Carlson & Lauder, 2011; Gerstner, 2007; Macdonnell, 1990; Witten & Hall, 2002).

An area of innovation that would benefit from a better understanding of loricariid oral-disk structure, function, and performance on a range of surfaces is biomimetic suction cup design. Biomimetic suction cups have already been modeled on the anatomical suction cups of other fishes, such as the ventral sucker of gobiesocid clingfishes (Ditsche et al., 2014; Wainwright et al., 2013). Ditsche et al. (2014) and Wainwright et al. (2013) found that *Gobiesox meandricus* adheres equally well on a variety of fouled substrates, but they focused on only one species. Comparative studies such as ours, which examine

functional morphologies in a phylogenetic context, are better suited to identifying specific lineages that are most highly specialized for a given functional challenge. Whereas *Gobiesox meandricus* may be an excellent jack-of-all-trades, species of *Astroblepus* and *Chaetostoma* may be better models for suction cups that must adhere to smooth, clean substrates, which are encountered more often in rivers than in intertidal zones (Domokos et al., 2014). Nevertheless, further experiments are needed to compare loricarioid and clingfish sucker performance on different substrates.

Although this study focused on the tissue composition of loricarioid oral disks, various additional features of the loricarioid mouth may make important contributions to its function as an attachment organ (Crop, Pauwels, Van Hoorebeke, & Geerinckx, 2013; Geerinckx, Brunain, et al., 2007; Geerinckx, Herrel, & Adriaens, 2011; Ono, 1980), and to the function of anatomical suction cups more generally. For example, loricarioids have paired channels just posterior to their maxillary barbels, which allow water to flow into the oral sucker while still allowing the fish to regulate adhesion of the suction cup.

Opening and closing of these channels is controlled via manipulation of the maxilla itself, to which the maxillary barbel is attached, with the barbel being integrated into the oral disk (Geerinckx, Brunain, et al., 2007). A similar channel-like structure was recently described in the suction cups of blepharicerid fly larvae (Kang, Johnston, van de Kamp, Faragó, & Federle, 2019), suggesting such channels make an important contribution to the optimal performance of anatomical suction cups more generally. Additional comparative studies would help identify how these structures are optimized for performance in the widely divergent habitats occupied by various evolutionary lineages.

## 5 | CONCLUSIONS

Neotropical suckermouth catfishes have evolved thick, collagenous lips in correlation with high flow and rocky environments on multiple occasions. These morphological specializations likely improve suction-based adhesion and station-holding by reducing the possibility of suction cup failure due to slipping, buckling, and/or an incomplete seal. Effective biomimetic suction cups based on the suckermouth catfish oral disk may be developed for high flow conditions using a rigid body and a tough but deformable edge.

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## AUTHOR CONTRIBUTIONS

**Noah Bressman:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; visualization; writing-original draft; writing-review and editing. **Jonathan Armbruster:** Conceptualization; data curation; formal analysis; methodology; resources; visualization; writing-review and editing. **Nathan Lujan:** Conceptualization; investigation; methodology; resources; writing-review and editing. **Imoh Udoh:** Formal analysis; investigation; visualization; writing-review and editing. **Miriam Ashley-Ross:** Conceptualization; methodology; supervision; writing-review and editing.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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