

## RESEARCH ARTICLE

# Anatomical basis of diverse jaw protrusion directionality in ponyfishes (Family Leiognathidae)

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## Abstract

Protrusion of the oral jaws is a key morphological innovation that enhances feeding performance in fishes. The mechanisms of protrusion and the basis of variation in its magnitude are well studied, but little attention has been paid to the functional morphology of protrusion directionality, despite wide variation among teleost species from slightly dorsal to strongly ventral. Ponyfishes (Leiognathidae) comprise a group of 52 species that exhibit striking diversity in the directionality of jaw protrusion, providing a promising system for exploring its underlying basis in a single clade. We examined the anatomical basis of protrusion directionality by measuring eight traits associated with the size and positioning of oral jaw bones. Measurements were made on cleared and stained specimens of 20 ponyfish species, representing every major lineage within the family. Species fell into three nonoverlapping clusters with respect to directionality including dorsal, rostral, and ventral protruders. A key correlate of protrusion direction is the anterior–posterior position of the articular–quadrate jaw joint. As the joint position moves from a posterior to a more anterior location, the orientation of the relaxed mandible rotates from an almost horizontal resting position to an upright vertical posture. Abduction of the mandible from the horizontal position results in ventrally directed protrusion, while the more upright mandible rotates to a position that maintains dorsal orientation. The resting orientation of the premaxilla and maxilla, thus, vary consistently with protrusion direction. Mouth size, represented by length of the mandible and maxilla, is a second major axis of variation in ponyfishes that is independent of variation in protrusion directionality.

## KEYWORDS

Acanthomorpha, articular–quadrate jaw joint, premaxillary protrusion, slipmouths

## 1 | INTRODUCTION

The capacity for premaxillary protrusion is a major innovation in the jaws of fishes that results in several enhancements of feeding performance (Gosline, 1980; Motta, 1984; Muller & Osse, 1978). Jaw protrusion facilitates greater dexterity during various biting and manipulation behaviors (Gibb, Staab, Moran, & Ferry, 2015; Konow & Bellwood, 2005) and results in a substantial increase in suction feeding performance

(Holzman, Day, & Wainwright, 2007; Lauder, 1980; Liem, 1980). During suction feeding, jaw protrusion plays a central role in rapidly positioning the mouth aperture close to the prey item, allowing the spatially restricted suction flow maximum exposure to the prey (Holzman, Day, & Wainwright, 2007). The capacity for premaxillary jaw protrusion has evolved several times in ray-finned fishes, including independent origins in two of the most successful radiations, Cypriniformes and Acanthomorpha (Wainwright, McGee, Longo, & Hernandez, 2015).

While suction feeding movements in most fishes extend the mouth aperture in a rostral direction, numerous species that feed on benthic prey extend the mouth ventrally (Chao & Musick, 1977; Gosline, 1984; Liem, 1979), while fewer protrude somewhat dorsally and approach their prey from below (Alexander, 1967a; Grobecker, 1983; Liem, 1967; Pietsch, 1978). The diversity in protrusion directionality has been recognized for some time in the ecomorphological literature because it relates strongly to where fish feed, but the functional morphology that underlies this variation is poorly known.

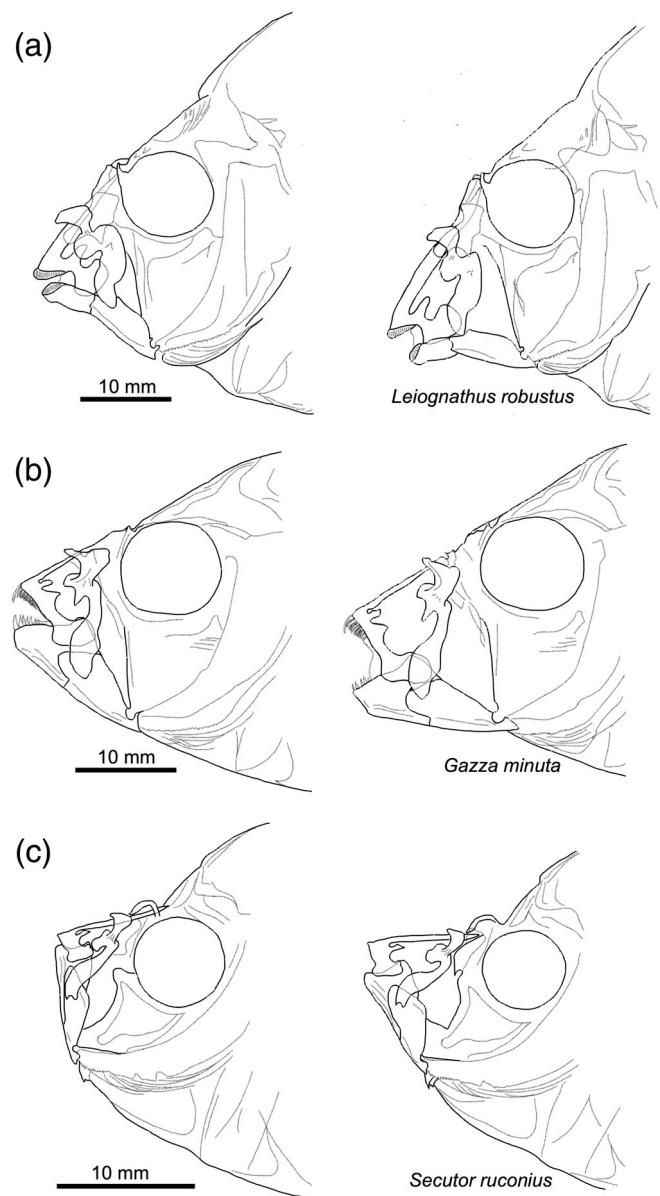
Ponyfishes (Family Leiognathidae) comprise a group of over 50 bioluminescent acanthomorph species that show striking diversity in jaw protrusion directionality (Figure 1). Their common names, “ponyfish” and “slipmouth,” refer to the horse-shaped cranial profile when the jaws are protracted and the great extent of this protrusion, respectively (Nelson, Grande, & Wilson, 2016). These fishes are found in sandy-shore habitats and open waters of the Indo-West Pacific. They feed on small benthic organisms and midwater zooplankton, with diet varying among species (Jing, 1997; Jones, 1985). Although extensively studied to understand their circumesophageal light organ system (Chakrabarty et al., 2011a; Harvey, 1921; Hastings, 1971; McFall-Ngai & Dunlap, 1984; Sasaki et al., 2003; Woodland, Cabanban, Taylor, & Taylor, 2002), less is known about their feeding mechanism (Jones, 1985). While their anatomical diversity has received some attention (Chakrabarty, Davis, Smith, Baldwin, & Sparks, 2011b; James, 1984; Jing, 1997; Jones, 1985; Seah, Abdullah, Zaidi, & Mazlan, 2009), no study has quantified the degree of variation in protrusion directionality among ponyfish species or examined the morphological differences underlying this variation.

Here, we determine the anatomical basis of protrusion directionality in leiognathids. We used clearing and staining, a procedure employed for visualizing the skeletal anatomy of small vertebrates, to prepare specimens of 20 species, spanning the range of jaw protrusion direction in ponyfishes. We then photographed the cleared and stained specimens and digitally measured craniofacial morphology. This effort allowed us to describe key anatomical features associated with protrusion direction and to explore other major axes of morphological diversity within a family that exhibits extensive craniofacial variation.

## 2 | MATERIALS AND METHODS

### 2.1 | Study specimens and anatomical measurements

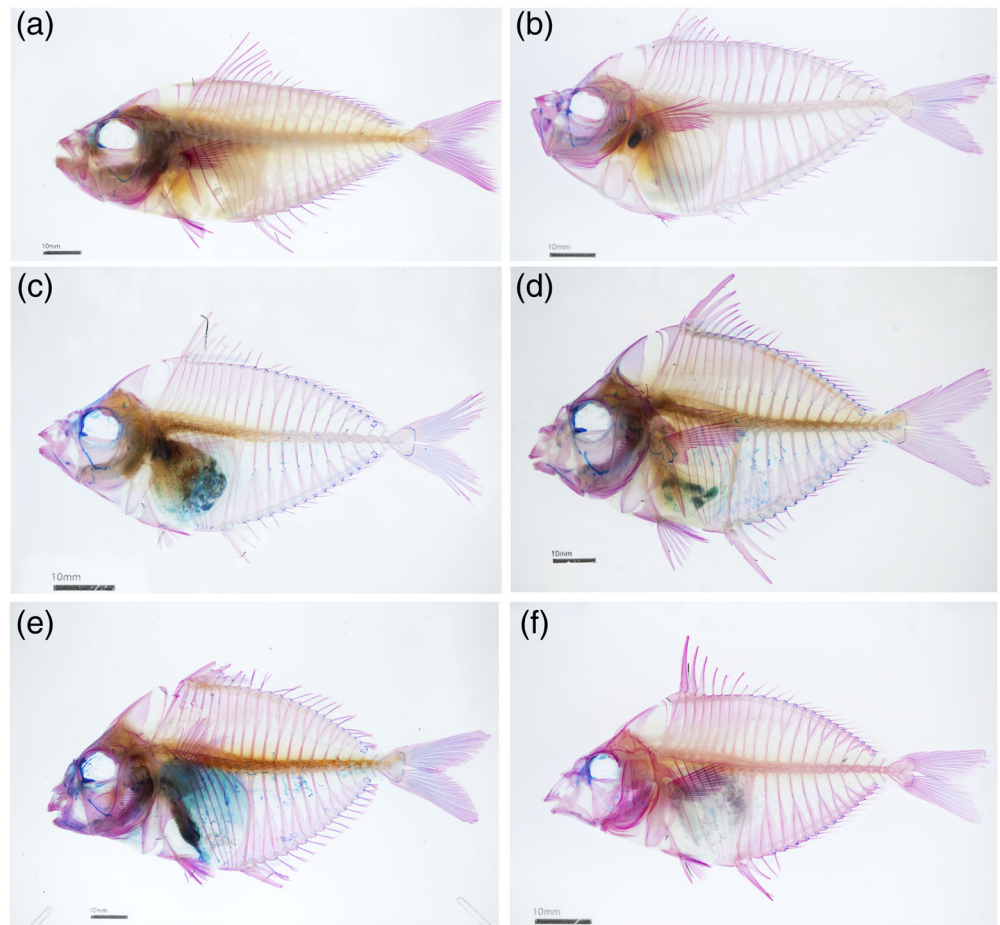
We examined 49 specimens representing 20 leiognathid species. Specimens were obtained from the Louisiana State University Museum of Natural Science and the Smithsonian National Museum of Natural History. Specimens were fixed in formalin and stored in 70% ethanol prior to this study (Table S1). Each specimen was cleared and stained using procedures adapted from Dingerkus and Uhler (1977) and Taylor and Van Dyke (1985). Cleared and stained specimens were stored in



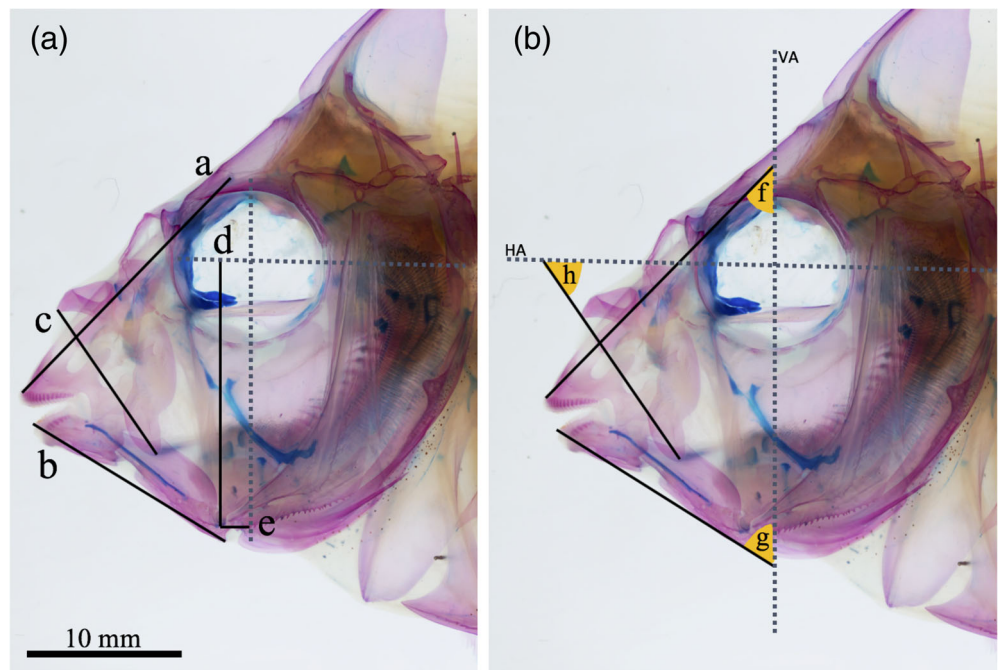
**FIGURE 1** Leiognathids show diversity in the orientation of oral jaw protrusion, with species that protrude their jaws in a ventral (a), rostral (b), or dorsal (c) direction. Left and right panels depicts specimens in a relaxed, mostly retracted posture and a protruded jaw position, respectively. Black bars indicate a 10 mm scale. Illustrations by A. Roberts

glycerin and the left side of each specimen was photographed according to procedures outlined in *Photographing and Measuring Cleared and Stained Specimens* (supplementary online material). We photographed specimens in a relaxed, mostly retracted posture (Figure 1, left panel; Figure 2) and after gently manipulating the jaws into a protruded position by abducting the mandible while fixing the position of the neurocranium (Figure 1, right panel). In the absence of published video data of ponyfish feeding, we guided these manipulations based on experience with a wide diversity of percomorph fishes for which we have collected videos and manipulated specimens (Longo, McGee, Oufiero, Waltzek, & Wainwright, 2016; Martinez, McGee,

**FIGURE 2** Cleared and stained ponyfishes depict variation in craniofacial morphology and body form. Species include *Gazza aklamys* (a), *Secutor insidiator* (b), *Photopectoralis aureus* (c), *Leiognathus robustus* (d), *Eubleekeria jonesi* (e), and *Nuchequula decorus* (f). Black bars indicate a 10 mm scale



**FIGURE 3** Measurements made on ponyfish jaw morphology, depicted with *Leiognathus robustus*. (a) Ascending process length (a), mandible length (b), maxilla length (c), and the vertical (d) and horizontal (e) positions of the jaw joint were measured digitally from photographs. (b) Angular orientations of the ascending process (f; in reference to the vertical axis), mandible (g; in reference to the vertical axis), and the maxilla (h; in reference to the horizontal axis) were measured from specimen photographs in the “at rest” mouth position. Dotted gray lines represent the horizontal (HA) and vertical (VA) axes



Borstein, & Wainwright, 2018; Oufiero, Holzman, Young, & Wainwright, 2012).

Using NIH ImageJ software (Schneider, Rasband, & Eliceiri, 2012), we made linear and angular measurements of morphological traits

from the photographs to characterize oral jaw and craniofacial anatomy when the jaws were in the relaxed, closed mouth position (Figure 3). Linear measurements included standard length, premaxillary ascending process length (a), mandible length (b), maxilla length

(c), and the vertical (d) and horizontal (e) positions of the articular-quadrant jaw joint (Figure 3a). The vertical position of the jaw joint was measured as the vertical distance between the jaw joint and the specimen's mid-horizontal axis (HA), which was defined as a line from the center of the orbit to the center of the caudal peduncle. The horizontal position of the jaw joint was measured as the horizontal distance between the jaw joint and a vertical axis (VA), drawn perpendicular to the horizontal axis and through the center of the orbit. Angular measurements included orientations of the premaxillary ascending process (f), mandible (g), and the maxilla (h; Figure 3b). The orientations of the premaxillary ascending process and mandible were measured with respect to the vertical axis; the orientation of the maxilla was measured with respect to the horizontal axis.

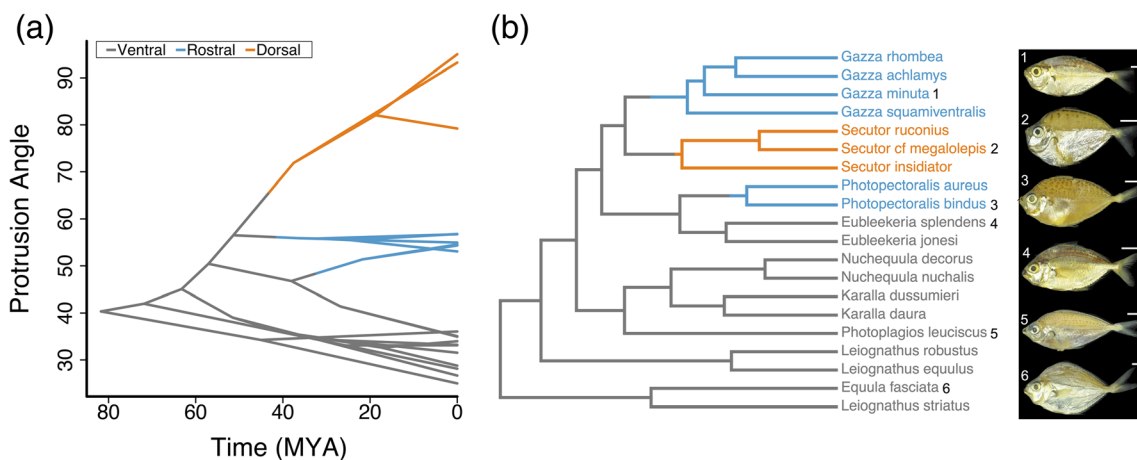
Ponyfishes have previously been assigned to ventral, rostral, or dorsal protruding functional groups based on differences between the “at rest” and “open” mouth positions, diet, and tooth shape (Jones, 1985). We contrasted these groupings with our measure of protrusion angle, defined as premaxillary ascending process orientation relative to the vertical axis when specimens were in the opened-mouth, protruded jaw position. This measurement confirmed that our sample of species fell into three nonoverlapping functional groups: ventral protruders with a protrusion angle of 28–39°; rostral protruders with a protrusion angle of 53–57°; and dorsal protruders with a protrusion angle of 76–92° (Figure 4). Of our 20 study species, 11 are ventral protruders, six are rostral protruders, and three are dorsal protruders resulting in ~50% sampling fraction for each functional group based on previous assignments (Jones, 1985).

## 2.2 | Anatomical and statistical comparisons of oral jaw functional groups

We computed species means for each morphological trait and log transformed mean values for linear traits to better approximate a

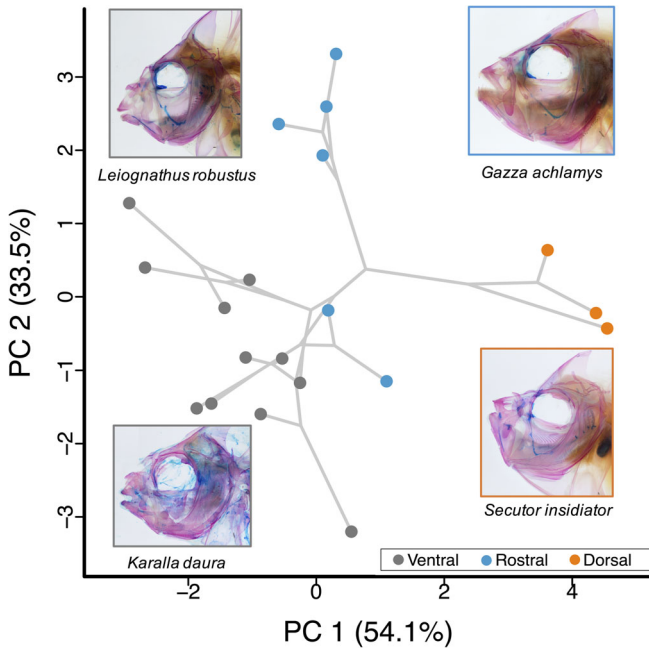
normal distribution of the data in R v. 3.5.1 (R Core Team, 2019). Using phylogenetic regression, we size corrected trait measurements that showed a significant relationship with standard length, including ascending process length, mandible length, maxilla length, and vertical position of the jaw joint. This correction was performed using packages *ape* (Paradis et al., 2018), *phytools* (Revell, 2018), and *geiger* (Harmon et al., 2019) and a trimmed version of the most comprehensive, time-calibrated ponyfish phylogeny (Chakrabarty, Davis, Smith, Baldwin, & Sparks, 2011b). For each species in the dataset, we captured residuals with the *phyl.resid* function under a Brownian Motion model for each size dependent trait. All eight measurements were then converted to standard normal deviates (i.e., z-scores) using the *scale* function (Becker, Chambers, & Wilks, 1988) to ensure that all measurements were in commensurate units and scales, thus producing a Euclidean morphospace for subsequent multivariate analyses (Adams & Collyer, 2019; Huttegger & Mitteroecker, 2011). These values were used for all subsequent analyses.

To visualize the multivariate morphological data, we performed a Principal Component Analysis (PCA) on the correlation matrix of all eight traits using the *prcomp* function. The resulting PC loadings were examined to determine how each morphological trait contributes to the variation along each PC axis. We used phylogenetic regressions to explore the pairwise relationships between traits and examined violin plots to explore trait variation within individual ponyfish functional groups. To test whether the functional groups have statistically different jaw and craniofacial morphology, we performed a phylogenetic multivariate analysis of variance (phylogenetic MANOVA) on the eight morphological traits over 10,000 iterations to test for statistical significance under a Brownian Motion model. This same method, implemented with the *procD.pgls* function (Adams, 2014; Adams & Collyer, 2015, 2016; Adams & Collyer, 2018a; Collyer, Sekora, & Adams, 2015) within the *geomorph* package (Adams, Collyer, & Kaliontzopoulou, 2019), was used to perform individual phylogenetic ANOVAs on each of the eight traits. Finally, we performed pairwise



**FIGURE 4** Jaw protrusion orientation in ponyfishes. (a) Phenogram of protrusion angle shows the clustering of dorsal, rostral and ventral protruding species. (b) Annotated phylogeny depicts the relationships between the 20 leiognathid species examined in this study. Numbers indicate species shown in right-hand images. White bars indicate a 10 mm scale

comparisons of mean trait values among functional groups (i.e., ventral–rostral protruders, ventral–dorsal protruders, and rostral–dorsal protruders) using the *pairwise* function (Adams & Collyer, 2018b; Collyer, Sekora, & Adams, 2015) in the *RRPP* package (Adams & Collyer, 2020). Resulting, pairwise *z*-values and *p*-values were evaluated to determine the magnitude and significance of the Euclidean distance between mean trait values of each functional group contrast, respectively.



**FIGURE 5** A phylomorphospace projection of ponyfish species based on eight measurements of jaw and cranial anatomy. Here, each point represents a species' standard normal deviation value, with ventral, rostral, and dorsal protruding species in gray, blue, and orange, respectively. Specimen images of outlier species are outlined according to their functional group. PC loadings are reported in Table 1

**TABLE 1** Principal component loadings on the first 5 PC axes.

Morphological trait	Principal component loadings				
	PC 1	PC 2	PC 3	PC 4	PC 5
Ascending process length	−0.40	0.29	−0.02	0.41	0.14
Mandible length	0.03	0.60	0.09	0.16	−0.25
Maxilla length	0.02	0.59	0.24	−0.16	−0.20
Ascending process orientation	0.43	0.22	−0.22	0.02	−0.34
Mandible orientation	−0.33	−0.28	0.71	0.07	−0.48
Maxilla orientation	0.44	0.03	0.43	−0.52	0.18
Horizontal jaw joint	0.43	0.02	0.42	0.62	0.45
Vertical jaw joint	−0.40	0.29	0.13	−0.36	0.54
Percent variance	54.1%	33.5%	6.9%	2.4%	1.6%
Cumulative variance	54.1%	87.6%	94.5%	96.9%	98.5%

Note: PC loadings indicate the contribution of each variable to the principal component. Bolded values represent loadings that are greater than or equal to |0.3|.

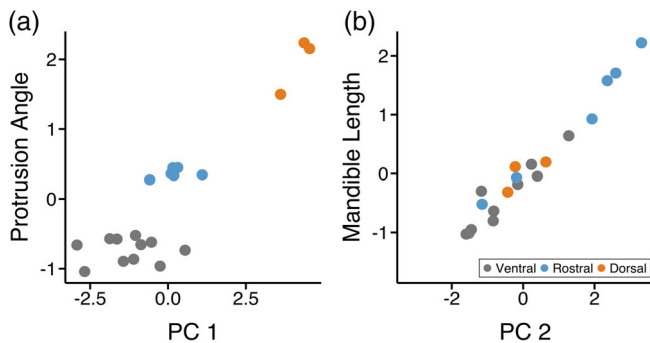
### 3 | RESULTS

#### 3.1 | Significant anatomical differentiation of functional groups

The first two axes of the PCA show some separation of functional groups in morphospace with PCs 1 and 2 describing 87.6% of variation in the data (Figure 5; Table 1). Ventral, rostral, and dorsal protruders group together along PC 1 from low to high values, respectively, suggesting that this axis primarily captures variation in species anatomy related to protrusion direction. A low value on this axis is associated with a long ascending process, a posteriorly positioned jaw joint, and the maxilla oriented with its distal arm directed posteriorly, while species with a positive position on PC 1 have a short ascending process, anteriorly and more dorsally positioned jaw joint, a more horizontally oriented ascending process, and an upright mandible (Figure 5). A phylogenetic regression of PC 1 scores against the standard normal deviates of protrusion angle confirms a strong relationship between this primary axis of morphological variation and our functional categorization of protrusion direction ( $p = 1.33^{-06}$ ; Figure 6a). Furthermore, individual phylogenetic regressions of protrusion angle against each of our eight traits reveal significant relationships with the horizontal position of the jaw joint ( $p = 1.04^{-02}$ ; Figure 7a), the vertical position of the jaw joint ( $p = 7.05^{-03}$ ; not pictured), premaxillary ascending process length ( $p = 1.54^{-03}$ ; not pictured), premaxillary ascending process orientation ( $p = 5.71^{-07}$ ; not pictured), mandible orientation ( $p = 5.80^{-03}$ ; Figure 7b), and maxilla orientation ( $p = 1.51^{-04}$ ; Figure 7c).

The lengths of the mandible and maxilla are strongly and positively correlated with PC 2, where  $p = 4.47^{-14}$  and  $1.24^{-11}$ , respectively (Figure 6b; Table 1). This PC axis separates the majority of species ( $n = 16$ ) from a group of four rostral protruding species, including *Gazza minuta*, *G. rhombea*, *G. squamiventralis*, and *G. achlamys*. Indicated by the strong, positive loading of the lengths of the upper and lower jaw bones on PC 2 (Table 1), these four species have a larger jaw apparatus than other ponyfishes. This pattern suggests a

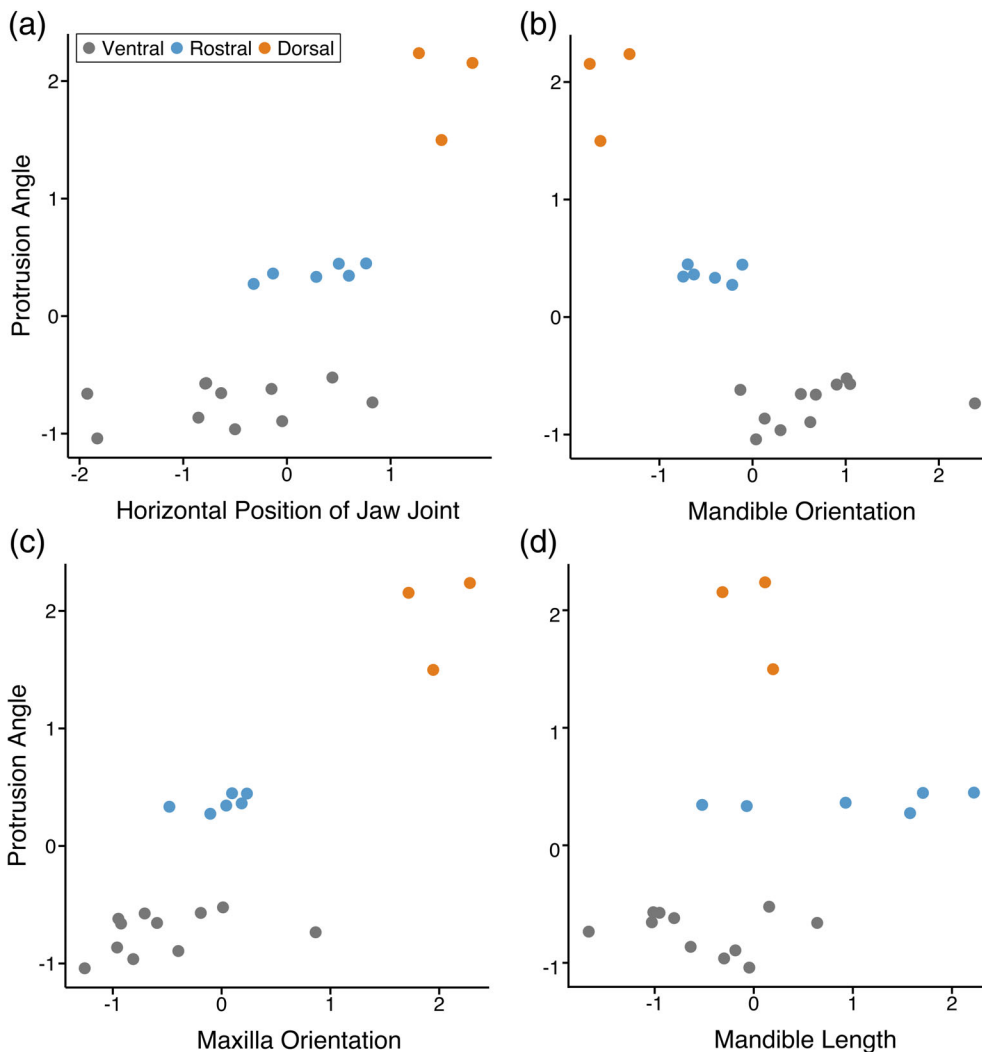
secondary axis of morphological variation in the size of the jaws and mouth. Phylogenetic regressions of mandible and maxilla length against the horizontal and vertical position of the jaw joint show a



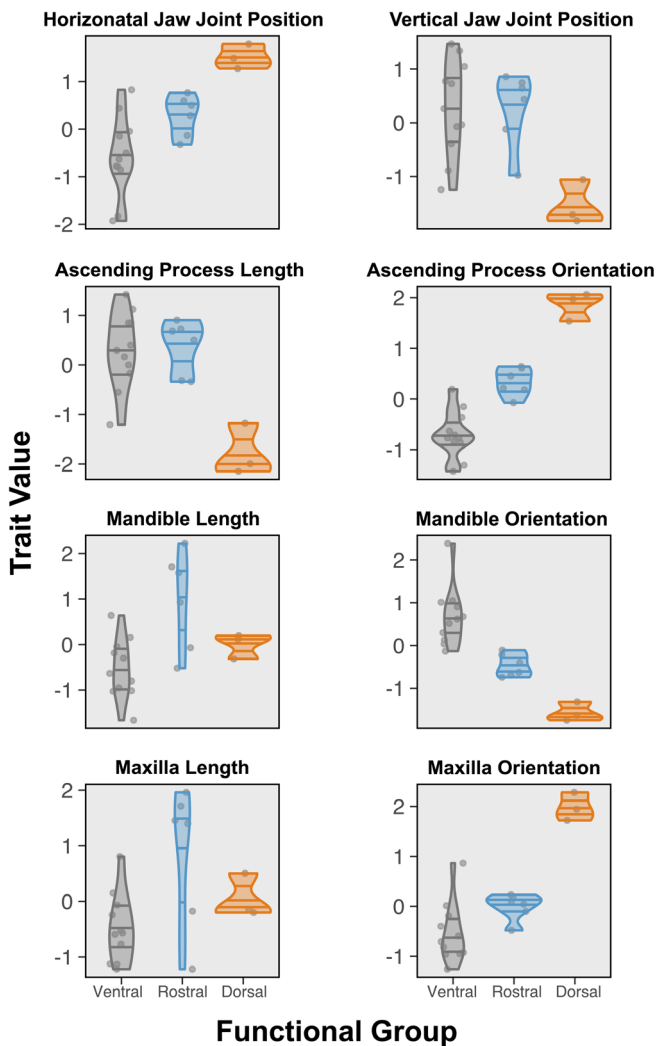
**FIGURE 6** Oral jaw protrusion direction and jaw size represent two major axes of morphological diversity in ponyfishes. Phylogenetic regression analyses of standard normal deviates reveal significant relationships between (a) protrusion angle and PC 1 ( $p = 1.33 \times 10^{-6}$ ; adjusted  $R^2 = 0.72$ ) and (b) mandible length and PC 2 ( $p = 4.47 \times 10^{-14}$ ; adjusted  $R^2 = 0.96$ )

significant, although weak, relationship between the vertical position of the jaw joint and maxilla length ( $p = .023$ ). However, all other comparisons between the jaw joint position and jaw length yielded insignificant relationships. In combination with the insignificant relationship between protrusion angle and mandible length ( $p = 0.49$ ; Figure 7d), these findings reinforce the PCA result that premaxillary protrusion direction (PC 1) and jaw size (PC 2) represent two independent axes of morphological variation in ponyfishes.

Violin plots suggest anatomical differentiation between oral jaw functional groups for all traits excluding mandible and maxilla length. Notably, the position of the jaw joint, premaxillary ascending process length, and all angles of orientation distinguish the dorsal protruders from the ventral and rostral protruders as their trait value distributions show little to no overlap (Figure 8). High variance seen in the violin plots of mandible and maxilla length for rostral protruders (Figure 8) reflects the diversity of rostral protruding species along PC 2 (Figure 5). Finally, violin plots show greater morphological variance among ventral protruding species (Figure 8), which is also apparent in the PCA as ventral protruding species occupy more area in the morphospace compared to rostral and dorsal protruding species (Figure 5).



**FIGURE 7** Phylogenetic regressions reveal significant relationships between protrusion angle and individual measurements of the jaw bones. Here, each point represents a species' standard normal deviation value with ventral, rostral, and dorsal protruding species in gray, blue, and orange, respectively. (a) Horizontal position of the jaw joint ( $p = 1.04 \times 10^{-2}$ ; adjusted  $R^2 = 0.27$ ), (b) mandible orientation ( $p = 5.80 \times 10^{-3}$ ; adjusted  $R^2 = 0.32$ ), and (c) maxilla orientation ( $p = 1.51 \times 10^{-4}$ ; adjusted  $R^2 = 0.53$ ) show a significant relationship with protrusion angle. In contrast, (d) protrusion angle and mandible length show a nonsignificant relationship ( $p = 0.49$ ; adjusted  $R^2 = -0.03$ ), with ventral and rostral protruding species exhibiting considerable variation in mandible length



**FIGURE 8** Violin plots show nonoverlapping distributions for several morphological traits. For each trait, three distributions are shown representing the morphological variance exhibited by each functional group. Each distribution has three horizontal lines indicating the mean, upper, and lower quartiles. Each trait plot uses standard normal deviates collected from specimens in the “at rest” mouth position

The phylogenetic MANOVA shows a significant overall difference between functional groups ( $p = 1.00^{-4}$ ). Phylogenetic ANOVAs of individual traits reveal significant differences between functional group means for all variables except length of the maxilla (i.e., seven of the eight traits; Table 2). Pairwise comparisons of functional groups for each trait reveal that ventral and dorsal protruding species are the most morphologically different from each other, showing significant differences for all traits excluding mandible and maxilla length. Rostral and dorsal protruders differ significantly in four traits, including premaxillary ascending process length and orientation, maxilla orientation, and vertical position of the jaw joint. Ventral and rostral protruders only differ significantly in mean trait values for mandible length and orientation, as well as premaxillary ascending process orientation. Effect size results (i.e., pairwise  $z$ -values) reveal which traits

are most statistically important in differentiating between functional groups as this analysis quantifies the magnitude of difference between mean trait values (Table 2). Here, all traits that had significant  $p$ -values ( $p \leq 0.05$ ) also had large effect sizes where  $z \geq |2|$ , indicating that functional group mean trait values are separated by at least two standard deviations. Premaxillary ascending process length and orientation, as well as maxilla orientation most distinguish ventral and dorsal protruders. Premaxillary ascending process length, maxilla orientation, and the vertical position of the jaw joint show the largest effect sizes between rostral and dorsal protruding species. Ventral and rostral protruding species show the strongest differences in mandible length and orientation, as well as premaxillary ascending process orientation.

## 4 | DISCUSSION

There is little known about the anatomical basis of jaw protrusion directionality despite considerable variation among extant fishes. While most suction feeding acanthomorph fishes open their mouth and protrude their jaws in a rostral direction, a number of demersal lineages that feed on benthic prey, including many sciaenids, mullids, callionymids, and gerreids, have ventrally protruding jaws. In this study, we explored the craniofacial anatomy of ponyfishes, a family exhibiting unusual variation in premaxillary protrusion orientation. Our anatomical comparison of the three oral jaw functional groups within Leiognathidae broadens our general understanding of the relationship between feeding morphology and premaxillary protrusion directionality during feeding. Overall, significant anatomical differentiation of functional groups suggested that the three angles of orientation, premaxillary ascending process length, and the position of the jaw joint discriminate the three oral jaw functional groups.

Despite extensive variation in protrusion directionality, the overall shape and orientation of the anatomical components examined herein suggest that the jaw protrusion mechanism is fairly similar across this group. Specifically, our observations and manipulations of cleared and stained specimens indicate that ponyfish protrusion involves a common perciform mechanism that is actuated by lower jaw depression (Alexander, 1967b; Liem, 1970; Schaeffer & Rosen, 1961). Ligamentous connections between the coronoid process of the mandible and the distal end of the maxillary shaft results in the distal region of the maxilla being rotated anteriorly as the mandible is depressed. As the maxilla rotates, the deep process on its proximal head presses against the ventral surface of the ascending process of the premaxilla. This pressure is resisted by (a) a ligamentous loop that passes over the ascending process which connects the right and left maxillary heads and (b) the posteriorly tapered shape of the ascending process with associated rostral cartilage. Thus, the premaxilla responds to this maxillary pressure by sliding anteriorly into the protruded position. This basic mechanism appears to operate in all of the ponyfish species we studied.

How then do the anatomical differences translate to variation in the direction of jaw protrusion in ponyfishes? One key appears to be

**TABLE 2** Phylogenetic analyses of variance

Trait	Phylogenetic ANOVA				
	F-value (df = 2,18)	p-value	Functional group comparison	Pairwise z-value	Pairwise p-value
Ascending process length	9.27	<b>0.002</b>	Ventral – Rostral	-1.35	0.983
			Ventral – Dorsal	3.66	<b>0.003</b>
			Rostral – Dorsal	3.72	<b>0.002</b>
Mandible length	4.29	<b>0.029</b>	Ventral – Rostral	2.82	<b>0.010</b>
			Ventral – Dorsal	-0.68	0.694
			Rostral – Dorsal	1.10	0.146
Maxilla length	2.11	0.146	Ventral – Rostral	1.89	0.054
			Ventral – Dorsal	-0.81	0.746
			Rostral – Dorsal	0.49	0.290
Ascending process orientation	29.95	<b>1.00 e-4</b>	Ventral – Rostral	2.23	<b>0.018</b>
			Ventral – Dorsal	4.96	<b>1.00 e-4</b>
			Rostral – Dorsal	2.31	<b>0.027</b>
Mandible orientation	8.32	<b>0.003</b>	Ventral – Rostral	2.25	<b>0.028</b>
			Ventral – Dorsal	3.48	<b>0.002</b>
			Rostral – Dorsal	0.91	0.179
Maxilla orientation	9.76	<b>0.003</b>	Ventral – Rostral	-0.02	0.440
			Ventral – Dorsal	4.06	<b>9.00 e-4</b>
			Rostral – Dorsal	3.12	<b>0.006</b>
Horizontal position of the jaw joint	3.91	<b>0.036</b>	Ventral – Rostral	1.04	0.163
			Ventral – Dorsal	2.74	<b>0.009</b>
			Rostral – Dorsal	0.98	0.176
Vertical position of the jaw joint	4.80	<b>0.020</b>	Ventral – Rostral	-0.34	0.560
			Ventral – Dorsal	3.04	<b>0.006</b>
			Rostral – Dorsal	2.34	<b>0.021</b>

Note: Phylogenetic ANOVAs were performed on the standard normal deviates of the eight morphological traits. Pairwise effect sizes (i.e., z-values) indicate the magnitude of the difference between functional group means, where values  $\geq |2|$  indicate that group means are separated by two or more standard deviations. Pairwise p-values indicate functional groups that exhibit significantly different morphologies for a specified trait. Significant p-values ( $\leq 0.05$ ) are bolded.

the position of the articular-quadrato joint. The anterior position of the joint in dorsal protruders results in an upright, nearly vertical position of the adducted mandible. A more posterior position of the jaw joint in ventral protruding species results in a nearly horizontal orientation of the adducted mandible (Figure 1). In our cleared and stained specimens, a moderate mandible abduction of about  $20^\circ$  results in a dorsally oriented mandible in the dorsal protruders, but a distinctly ventral orientation in the ventral protruders. In contrast to ventral and dorsal protruding species, rostral protruders have an intermediate jaw joint position and orientation of the resting mandible. Thus, the position of the jaw joint along the anterior–posterior axis of the fish strongly affects the resting orientation of the mandible, setting the boundary for mandibular rotation and, in turn, the protrusion angle of the upper jaw.

While the position of the jaw joint appears to play a key role in determining protrusion directionality, other variables contribute to the strong relationship between PC 1 and protrusion direction (Figure 6a; Table 1). The distal end of the maxillary arm is more anteriorly oriented at rest in dorsal protruders and posteriorly oriented in ventral

protruders (Figures 1, 2, & 7c). Also, the premaxillary ascending process is more vertically oriented in the ventral protruders and almost horizontally in dorsal protruders at rest (Figures 1 & 2). Finally, ventral protruders have the longest ascending process and dorsal protruders the shortest (Figures 1, 2, & 8).

Our results also reveal that jaw and mouth size represent a second major axis of variation among ponyfish species. Our findings show that PC 1 correlates strongly with variation in protrusion directionality while PC 2 primarily reflects differences in jaw size, indicating that these traits represent independent axes of diversity in this group. As proxies for jaw size, maxilla and mandible length show considerable variation within ventral and rostral protruding species but have little effect on protrusion directionality (Figure 7d). The length of the maxilla, length of the mandible, and location along the mandible where these two bones are connected form a large portion of the circumference of the gape when the jaws are extended. Thus, we propose that changes to this combination of traits results in differences in overall gape size.



Variation in protrusion directionality and jaw size in leiognathids is likely associated with differences in feeding ecology similar to the ecomorphological patterns seen in other fish groups (Chao & Musick, 1977; Gosline, 1984; Liem, 1967; Liem, 1979). Though ponyfish diets are taxonomically diverse and vary among species, some patterns emerge with respect to protrusion directionality. The diet of ventral protruding species comprises benthic prey, including infaunal polychaetes, mollusks, harpacticoid copepods, and a substantial contribution of planktonic calanoid copepods, crab larvae, and other midwater prey (Hajisamae, Chou, & Ibrahim, 2004; Kanou, Sano, & Kohno, 2004; Kuthalingham, 1958; Tham, 1950; Tiews, Divino, Ronquillo, & Marquez, 1968). Some rostral protruding species have been found to feed on other fish, polychaetes, and copepods (Blaber, 1980; Jing, 1997; Seah, Abdullah, Zaidi, & Mazlan, 2009). Dorsal protruding species are recorded as primarily feeding on small midwater plankton, including calanoid copepods, and fish eggs (Blaber, 1980; Tiews, Divino, Ronquillo, & Marquez, 1968; Venkataraman, 1960). Thus, while the diversity of feeding habits among ponyfishes deserves additional attention, a substantial literature does indicate both extensive dietary overlap among species (Seah, Abdullah, Zaidi, & Mazlan, 2009; Tiews, Divino, Ronquillo, & Marquez, 1968), as well as a tendency for ventral, rostral, and dorsal protruders to feed at progressively more elevated positions in the water column (Jing, 1997; Jones, 1985).

## 5 | CONCLUSIONS

Unusual diversity of protrusion directionality among ponyfishes is based on variation in the location of the articular-quadrato jaw joint, combined with the size and positioning of the upper and lower jaw bones. The overall size of the jaws, indicated by length of the mandible and maxilla, varies independently of the direction of protrusion. Our study suggests two important areas for future investigation. First, our estimates of jaw function, especially directionality of protrusion, should be confirmed with kinematic analyses of live feeding videos. Second, while ponyfishes show unusually wide variation in protrusion angle among closely related species, there is considerable diversity in this trait across acanthomorph and cypriniform fishes. A key goal of future research will be to determine whether variation in protrusion direction is commonly associated with the same anatomical features found in ponyfishes. Anterior–posterior variation of the position of the articular-quadrato jaw joint may be a widespread anatomical mechanism for adjusting the directionality of jaw protrusion in teleosts, but it remains to be seen whether other groups have evolved different traits to facilitate this underappreciated aspect of diversity in fish feeding functional morphology.

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**Alexus Roberts:** Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing-original draft; writing-review and editing. **Jennifer Hodge:** Formal analysis; investigation; methodology; writing-review and editing. **Prosanta Chakrabarty:** Resources; writing-review and editing. **Peter Wainwright:** Conceptualization; formal analysis; resources; writing-original draft; writing-review and editing.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/jmor.21314>.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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## SUPPORTING INFORMATION

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