Re-Evaluation of Batoid Pectoral Morphology Reveals Novel Patterns of Diversity Among Major Lineages

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ABSTRACT Batoids (Chondrichthyes: Batoidea) are a diverse group of cartilaginous fishes which comprise a monophyletic sister lineage to all neoselachians or modern sharks. All species in this group possess anteroposteriorly expanded-pectoral fins, giving them a unique disc-like body form. Reliance on pectoral fins for propulsion ranges from minimal (sawfish) to almost complete dependence (skates and rays). A recent study on the diversity of planform pectoral fin shape in batoids compared overall patterns of morphological variation within the group. However, inconsistent pectoral homology prevented the study from accurately representing relationships within and among major batoid taxa. With previous work in mind, we undertook an independent investigation of pectoral form in batoids and evaluated the implications of shape diversity on locomotion and lifestyle, particularly in the skates (Rajoidei) and rays (Myliobatoidei). We used geometric morphometrics with sliding semilandmarks to analyze pectoral fin outlines and also calculate fin aspect ratios (AR), a functional trait linked to locomotion. In agreement with previous work, our results indicated that much of the evolution of batoid pectoral shape has occurred along a morphological axis that is closely related to AR. For species where kinematic data were available, both shape and AR were associated with swimming mode. This work further revealed novel patterns of shape variation among batoids, including strong bimodality of shape in rays, an intermediate location of skate species in the morphospace between benthic/demersal and pelagic rays, and approximately parallel shape trajectories in the benthic/demersal rays and skates. Finally, manipulation of landmarks verified the need for a consistent and accurate definition of homology for the outcome and efficacy of analyses of pectoral form and function in batoids. J. Morphol. 277:482–493, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: geometric morphometrics; pectoral fin; stingray; skate

INTRODUCTION

Batoids (Chondrichthyes: Batoidea) comprise a diverse group of cartilaginous fishes that includes skates, rays, sawfishes, and guitarfishes. With over 600 species (Aschliman et al., 2012), they occupy a range of environments from the deep ocean to riverine systems. Prevailing hypotheses of the interrelationships of batoids and their placement relative to other neoselachians have long been debated (Compagno, 1999; Winchell et al., 2004; Kriwet et al., 2009). Recent work by Aschliman et al. (2012) has improved understanding of relationships among major lineages, although questions still remain (e.g., subfamilial relationships, especially within Rajidae). Owing to their relatively basal position in the vertebrate tree of life, batoids (and cartilaginous fishes as a whole) provide valuable context for understanding modes of diversification within a group of organisms that includes all modern fishes and tetrapods (Dahn et al., 2007; Pradel et al., 2014).

A common feature of batoid fishes is anteroposteriorly expanded pectoral fins that are fused to the body, creating a disc-like body shape (except in species with shark-like bodies, like the sawfishes). While batoid pectoral fins are highly derived, they are supported by the same basic components shared with other elasmobranch fishes. In all batoids, homology of pectoral fins is clearly defined by numerous supporting radials that are attached at their proximal ends to larger basal radials, whose number vary by group, and which themselves originate at the scapulocoracoid or pectoral girdle. In most species, the neurocranium, jaws, synarcual,
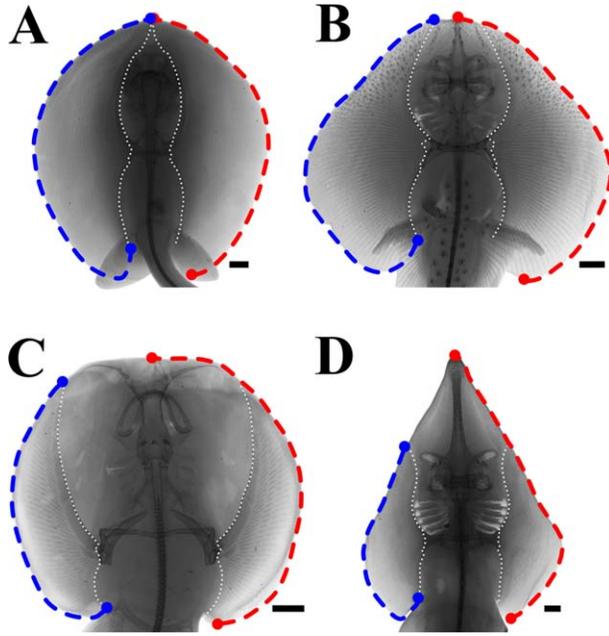
Fig. 1. Radiographs with examples of pectoral fin outlines used in this study (blue lines) versus that of Franklin et al. (2014) (red lines) for representative (**A**) Myliobatoidei (*Taeniura lymna*, AMNH 259883), (**B**) Rajoidei (*Leucoraja garmani*, AMNH 086127), (**C**) Torpedinoidei (*Torpedo torpedo*, AMNH 346), and (**D**) guitarfish (*Rhinobatos cemicula*, AMNH 49557). White dotted lines represent internal boundaries of pectoral fins, defining the medial limits of fin radial coverage in each. Scale bars = 1 cm.

trunk vertebrae (i.e., not including tail), and pelvic girdle are located medially, within the anteroposterior domain of the pectoral fins. Although the anterior margin of the batoid disc appears superficially similar among taxa, different skeletal components may support this region. In guitarfishes and most rajoids (skates), a rigid rostrum extends anteriorly from the neurocranium, with its distal end setting the anterior-most point of the disc. In these fishes, the anterior extent of the pectoral fin may fall well posterior to the distal end of the rostrum (Fig. 1). In contrast, the myliobatoids (stingrays) lack a rostrum and the anterior margin of the disc is defined by medially converging pectoral radials. The distinction is important, with implications for prey handling and feeding ecology (Wilga et al., 2012).

Among batoids, lifestyle and habitat diversity is greatest in the rays (Myliobatoidei), where some taxa are benthic or demersal and others are fully pelagic. Hydrodynamic conditions differ greatly between the sea floor and water column, requiring disparate suites of characteristics for life in each. For pelagic rays, the ability to create lift is necessary to control position in the water column, a critical quality as batoids lack swim bladders for fine-scale buoyancy control (Rosenberger, 2001). Benthic rays, on the other hand, rely more on maneuverability to navigate a structured environment (even sandy flats have much more structure than the open sea). Another major batoid clade,

the skates (Rajoidei), contains no fully pelagic species and has been described as an overall morphologically conserved clade (McEachran and Dunn, 1998) despite being the most species-rich group of elasmobranchs (Chiquillo et al., 2014). Basic knowledge on many skates is lacking due to the recent and rapid discovery of many species in remote, largely understudied regions (Aschliman et al., 2012). Consequently, the extent of locomotor and lifestyle diversity in rajoids relative to other batoids may be currently underestimated.

Variation in swimming mode is prominent among batoids and is related to both habitat and lifestyle diversity (Rosenberger, 2001; Wilga and Lauder, 2004). Batoid locomotion is divided into two major types, axial-based and pectoral-fin-based (Rosenberger, 2001). Axial locomotion relies either partially (some guitarfishes) or fully (guitarfishes, Pristidae, and Torpedinoidei) on undulations of a muscular trunk and tail to power propulsion (Shadwick and Gemballa, 2006). In contrast, the two most diverse batoid groups (Myliobatoidei and Rajoidei) primarily use pectoral fins as their source of propulsion. Among the pectoral-fin-based swimmers, species occur along a gradient from oscillation of the fin (i.e., flapping) to undulation within the fin, which is quantitatively defined by the number of waves passing along the pectoral fin margin during locomotion (Rosenberger, 2001). A wave number of one has been used as the threshold separating swimming styles, above which a species is described as having undulatory locomotion, and below oscillatory (Rosenberger, 2001). Benthic and demersal batoids tend to have relatively more pectoral fin waves, and pelagic species less.

Daniel (1988) showed that depending on fin wave number and fluid properties, there are fin shapes (i.e., aspect ratios) that theoretically minimize the cost of transport for the individual. Within this framework Daniel also suggested that higher aspect ratios are expected to correspond with fins that produce smaller wave numbers and have higher structural rigidity, patterns that have since been confirmed in empirical studies (e.g., Rosenberger, 2001; Schaefer and Summers, 2005). Both theoretical and observational evidence suggest clear links between the batoid pectoral form, its functionality and the lifestyle of the organism.

Until recently, patterns of morphological diversity in batoids had not been comprehensively assessed. However, two independent studies evaluated variation in batoid pectoral fins through examination of aspect ratio and fin cross-section (Fontanella et al., 2013) and also shapes of planform pectoral outlines, analyzed with geometric morphometrics (Franklin et al., 2014). Both showed that a major feature of batoid morphology concerns variation between rounded (low aspect ratio) forms and highly angular (high aspect ratio) forms, and that this variation was related to swimming mode. While Fontanella

et al. (2013) focused primarily on identifying differences between batoids with oscillatory and undulatory swimming styles, Franklin et al. (2014) provided a more detailed set of comparisons based on both swimming categories and taxonomic groupings. However, inconsistent homology used by Franklin et al. (2014) introduced nonpectoral shape variation that puts into question their results concerning relative diversity and patterns of morphospace occupation for batoid lineages. The primary issue arose from the incorrect placement of geometric landmarks in the anterior outline of the pectoral fin, as defined previously. A conflation of the rostrum and other nonpectoral structures with the anterior tip of the pectoral fin led to varying degrees of departure from true pectoral shape in different batoid clades (see Fig. 1 for examples). Therefore, morphological relationships among major batoid taxa were not accurately represented. Other landmark issues and treatments of morphological data (discussed further) appear to have also influenced their results. As a consequence, additional work is necessary to resolve patterns of shape diversity within the batoids.

The primary aim of this work was to reassess overriding patterns of morphological diversity for a prominent feature of the batoid bauplan, their highly modified pectoral fins, in order to shed light on processes of evolution and diversification within the group. In doing so, we pay special attention to the two taxa with pectoral-fin-based locomotion, Rajoidei and Myliobatoidei, whose similar disc-shaped bodies were recently suggested to have been achieved independently through convergence (Aschliman et al., 2012). We suggest that rajoids have not been fully appreciated as the morphologically (and ecologically) diverse group that they are and also that they have evolved quite a similar pattern of pectoral diversity to that of the myliobatoids minus the morphologically extreme pelagic forms. In order to assess shape diversity in rajoids and myliobatoids, we explored patterns of morphospace occupation. To do this we analyzed shape disparity, the morphological distance between groups and directionality of shape variation. We predicted that despite often being labeled as morphologically conservative, the amount of shape variance within the species-rich rajoid clade would rival that of the nonpelagic myliobatoids. In addition, the directionality of shape variation in rajoids and benthic/demersal myliobatoids would be similar in nature, reflecting evolution for life in structurally comparable habitats. We were also interested in potential functional implications of batoid shape diversity observed in this study and in comparing these results with that of previous studies. Pectoral fin aspect ratio (AR) was used as an indicator of functionality and we examined its variation in different groups to make inferences of potential swimming and lifestyle diversity. We used a molecular phylogeny from Aschliman et al. (2012) to compare rates of

overall shape and AR evolution in a subset of batoid species. Given fairly similar ages of rajoid and myliobatoid groups (Aschliman et al., 2012), we expected that rates in rajoids would be less than that of myliobatoids due to the existence of morphologically distinct pelagic forms in the latter, but more comparable to the benthic/demersal myliobatoids. Finally, in light of the recent work by Franklin et al. (2014), we manipulated pectoral landmark configurations to scrutinize the homology of the batoid disc.

MATERIAL AND METHODS
Specimen Image Collections

We collected specimen photographs for a number of batoid taxa, spanning much of the taxonomic and morphological diversity exhibited within the group. A total of 217 images were acquired, representing 41 stingray species from 21 genera (Myliobatoidei), 53 skate species from 21 genera (Rajoidei), 6 torpedo ray species from 4 genera (Torpedinoidei), 5 guitarfish species from 3 genera (undefined taxonomic relationship), 1 thornback ray (Platyrhinoidei) and 1 sawfish (Pristidae, but considered a derived guitarfish). For this work, guitarfish groupings follow Aschliman et al. (2012), with two clades termed "guitarfish 1" and "guitarfish 2" that includes Pristidae. In addition, rajoids were considered as a single family, Rajidae, as inter-relationships of subtaxa are not stabilized (John McEachran, personal communication). Specimen images came from numerous sources (for full list of specimens used see Supporting Information Table S1), including personally photographed fishes from the American Museum of Natural History (AMNH) and others collected under the Institutional Animal Care and Use Committee (IACUC) 2012-1994-NF-10.12.15-FI at Stony Brook University. In all cases, caution was used to include specimens where the body was in good enough condition to reasonably discern the outer margin of the disc. All photographs but one (*Paratrygon aiereba*, AMNH 59865) were taken from a dorsal view, with pectoral fins spread on a flat surface. Scale bars were also included in all images in order to scale for size in subsequent comparisons of shape variation. In some instances, ethanol-preserved specimens exhibited curving of the distal regions of the fin. When this occurred, magnets were used to keep fins on a flat plane, ensuring a natural resting orientation. Effort was made to primarily include adult specimens, but in some instances (e.g., very large species) smaller individuals were used. We used both male and female individuals, although sexual dimorphism does occur in some batoid species (e.g., Martinez et al., 2016). However, inspection of the major axes of variation did not indicate that sex influenced overall interpretations.

Shape Acquisition and Major Axes of Pectoral Variation

Pectoral fins were defined as the area supported by plesodic radials (Fig. 1). The anterior and posterior endpoints of fin outlines were discernible in most images, but in some cases we also consulted radiographs or specimen collections. We used geometric morphometrics to analyze variation in fin shapes. This approach uses a landmark-based registration of shape to compare specimens in multidimensional shape space (Zelditch et al., 2004). A total of 35 points were placed along the outer margin of the left pectoral fin (the right fin was occasionally used if the left was damaged), with 33 sliding semilandmarks and 1 fixed landmark on each end. Fixed (=type 1) landmarks are allowed to vary in two dimensions and mark homologous and identifiable points on all specimens (Bookstein, 1991). In comparison, semilandmarks are constrained to occur along a tangent line between adjacent curve points. In this way, they collectively capture the outline of a homologous structure, where individual fixed points

may be difficult to identify. Thirty-five total landmarks (fixed and semilandmarks) were included to reduce the number of shape variables produced, while providing an appropriate fit to the pectoral outline. Points along outlines were captured with the program tpsDIG2 (Rohlf, 2013), creating approximately evenly spaced points along the perimeter of the fin. Special landmark placement was needed for the lesser devil ray (*Mobula hypostoma*, AMNH 98192) because its anterior pectoral radials are incorporated into a highly modified cephalic lobe that curves into a third dimension. Here, anterior landmarks were placed along the margin of the neurocranium to the midline of the fish, in similar fashion to placement on other rays.

Semilandmarks were first slid to minimize the sum of squared landmark distances and the resulting configurations were then superimposed using generalized Procrustes analysis (GPA) in the *geomorph* package (Adams and Otarola-Castillo, 2013) in R (R Core team, 2013). Generalized Procrustes analysis scales, rotates and translates landmarks to reduce the sum of squared residuals of Procrustes distances between specimen shapes and an average or consensus shape (Rohlf, 1999; Klingenberg, 2010). Because we had multiple specimens for some species, we computed average shapes of species in order to avoid bias due to unequal sampling. A total of 106 shapes remained, each representing a single batoid species. As an initial examination of shape variation, we performed a principal component analysis (PCA) on the averaged specimens. The PCA provided a low-dimensional approximation of multivariate shape space, capturing maximum variance among a set of orthogonal axes (Zelditch et al., 2004). We also performed separate PCAs for Rajoidei and benthomorph rays (see below) to get a better indication of shape variation within each of the groups.

Evaluation of Batoid Morphospace

We evaluated morphospace occupation of pectoral-based swimmers, rajoids and myliobatoids, with comparisons of shape disparity, morphological distance and directionality of variation. We did not include the axial-based swimmers due to limited sampling and because many of them were represented by relatively few species. All comparisons were based on two grouping schemes, the first simply a comparison of rajoids versus myliobatoids (96 species). A second set of comparisons again included rajoid and myliobatoid species, but with the latter further divided into two subgroups that represented a practical construct based on the presence of distinctly separated morphological groups in the suborder. The first group, "pelagomorphs" (11 species), contained most of the pelagic myliobatoids (Mobulidae, Myliobatidae, and Rhinopteridae) but also the demersal Gymnuridae. The "benthomorphs" (30 species) contained the remainder of the rays (Dasyatidae, Plesiobatidae, Potamotrygonidae, Urolophidae, and Urotrygonidae), and also included the pelagic stingray (*Pteroplatytrygon violacea*). Aside from the two noted exceptions, these new morphological groups corresponded well with habitat use within the myliobatoids. Although the categorization was ad hoc, it was necessary to accurately represent the shape variation of myliobatidae, which showed clear signs of bimodality due to a large morphological gap between pelagomorphs and benthomorphs. In other words, treating myliobatoids solely as a single morphological group would be a mischaracterization of the shape diversity in the suborder and would also create an unfair comparison against rajoid diversity.

Shape disparity was calculated as the Procrustes variance of shapes within suborders and morphotypes, while morphological distances between group means were determined simultaneously with Procrustes ANOVA. Both tests were implemented in *geomorph* and, with significance based on 10,000-replicate randomizations of species relative to group designations. Finally, we compared the directionality of shape variation within rajoids and myliobatoids with visualization of shape changes in each group and calculations of slopes in PC space.
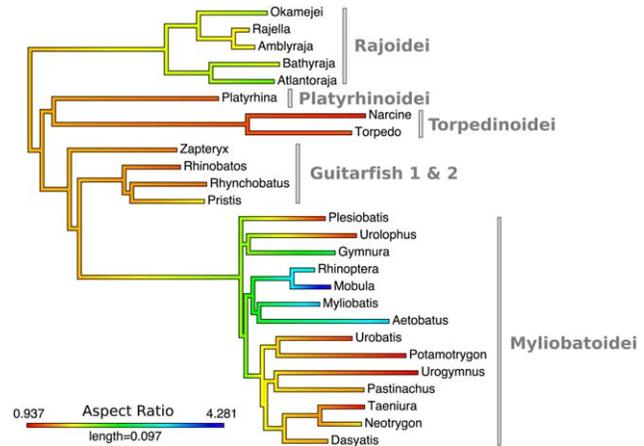

Fig. 2. Batoid phylogeny from Aschliman et al. (2012), trimmed to show only taxa overlapping with this study. Maximum likelihood (ML) state reconstruction of aspect ratio (AR) is mapped onto the tree.

Functional Implications of Pectoral Shape

We investigated the relationship between pectoral fin shape and aspect ratio (AR). Aspect ratio was calculated as the maximum chord width of the pectoral fin squared, divided by its surface area (Supporting Information Fig. S1). Aspect ratio measurements were doubled for the single calculated fin to get the estimated value for both fins (Wainwright et al., 2002). As with landmark placement, we did not include the cephalic lobe of *Mobula hypostoma* in aspect ratio calculations. We regressed pectoral shape data on AR for all species in *geomorph*, with a 10,000-iteration resampling procedure. We also performed a regression for just the pectoral-fin-based swimmers (rajoids and myliobatoids). Additionally, we tested for differences in regression slopes between rajoids and myliobatoids and between rajoids and benthomorph myliobatoids. Last, we regressed principal component (PC) scores on AR.

To account for phylogenetic nonindependence of observations, we also used comparative methods to test the relationship between shape and aspect ratio in a subset of species. We used a batoid phylogeny published by Aschliman et al. (2012; Fig. 2), which was based on Bayesian analysis of both mitochondrial and nuclear genes. We were limited by the overlap of taxa used in our study and in Aschliman et al. (2012). When there were both molecular and morphological data for a given species, we used that species to represent its genus. For cases where morphological and molecular data overlapped at the genus level but species did not match, we calculated average shapes and aspect ratios in all species that we did have for the genus in question. When this occurred, we insured that morphologies used to compute the average were not radically different from the species from which the molecular data came. In total, there were 26 genera available for comparison. We used *geomorph* to perform a phylogenetic generalized least squares regression (PGLS) to compare pectoral shape and AR under a Brownian motion model of trait evolution. Significance was assessed from 10,000-replicate permutations of shape across branch tips. In order to visualize changes in AR across the batoid phylogeny, we also used the *ape* package (Paradis et al., 2004) in R to reconstruct ancestral AR states with maximum likelihood.

We also compared swimming mode, defined by mean numbers of waves on pectoral margins during locomotion, to PC scores (as in Franklin et al., 2014) and also to AR. This was done to confirm that shape variation of pectoral fins was indicative of locomotor strategies and could, therefore, be used to infer swimming and lifestyle diversity in batoids. Fin wave data came from kinematic analyses of locomotion in nine batoid species (Rosenberger, 2001), of which we had shape and AR data for eight. For the ninth,
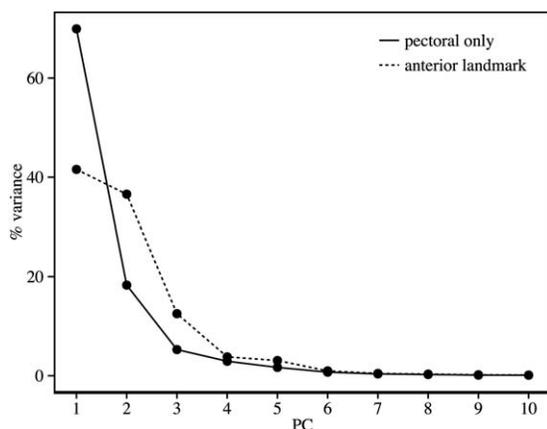
Fig. 3. Scree plot from PCAs of aligned shapes of all batoids used in this study. Results are shown for the original outline configuration (solid line) and the configuration with an additional anterior landmark (dashed line). For visualization, only the first 10 PCs are provided.

Rhinobatos lentiginosus, we used average PC scores and AR values from two other species in the genus *Rhinobatos*.

Rates of Shape and AR Evolution

Evolutionary rates of pectoral shape and AR change were estimated for groups of batoids, with taxa again limited to those overlapping between this study and that of Aschliman et al. (2012). This procedure, performed in *geomorph*, calculates rates of evolution under a Brownian model, with significance determined by phylogenetic simulation. As before, two sets of comparisons were made between rajoids and myliobatoids and also between rajoids, pelagomorph, and benthomorph myliobatoids. Additionally, we compared rates between benthic/demersal and fully pelagic taxa, irrespective of previous groupings (i.e., strictly habitat-based).

Homology of Pectoral Fin Landmarks

We performed a second set of morphological analyses to address a question of pectoral fin homology that arose from recent work on batoid form (Franklin et al., 2014). To understand the impacts of this definition of pectoral shape, we added a fixed landmark at the anterior point of the disc to the configuration of landmarks used in this study. Because we used neither the exact shape definition as in Franklin et al. (2014) nor the same taxa, we did not expect to recover the same results as their work. Rather, we sought to identify the effect of the anterior disc landmark when added to the consistently homologous pectoral fin outlines assessed in this study.

For comparison with our previous results, we also performed a PCA on the aligned shapes and did a regression of shape on AR. Following Franklin et al. (2014), we specified the anteriormost landmark for Pristidae (*Pristis pectinata*) at a medial position along the anterior margin of the neurocranium and not the end of its derived rostral saw (Fig. 1D). In addition, we did not include the rostral tendril on *Anacanthobatis folirostris*.

RESULTS
Major Axes of Pectoral Variation

A scree plot suggested that the amount of variance accounted for by the principal components (PCs) leveled off near the third axis (Fig. 3). The first two PCs accounted for 88.24% of total Batoid pectoral shape variation, with 69.94% attributed to PC 1 alone. On these axes, batoids displayed clustering patterns such that pectoral-fin-based swimmers displayed three distinct groups: pelagomorph myliobatoids, benthomorph myliobatoids, and rajoids.

Much of the overall shape variation occurred along a gradient from rounded to laterally expanded, triangular forms (PC 1 in Fig. 4). The largest PC 1 values included pelagic Myliobatidae and demersal Gymnuridae, with all remaining benthic and demersal families occurred at much smaller values (Plesiobatidae, Potamotrygonidae, Urolophidae, and Urotrygonidae). Dasyatidae was also oriented toward small PC 1 scores, but covered a large range that went into intermediate scores on the axis. The rajoids were centered on intermediate PC 1 values, displaying a large range on that axis. All of the axial-based swimmers (Narcinidae, Pristidae, Rhinobatidae, and Torpedinidae) occurred in negative regions of PC 1.

The second PC was associated with variation in the location of the pectoral fin's lateral apex that effectively divides the fin into anterior and posterior regions. Rajoids displayed the largest variation on this axis relative to other batoid groups and along with the guitarfishes, also set its upper limits. These species had pectoral fins that were increasingly asymmetrical, with a posteriorly oriented lateral fin apex, creating a convex or lobed posterior fin. In comparison, the benthic rays had lower PC 2 values with more anteroposteriorly symmetrical fins. The third PC accounted for 5.28% of total shape variance (93.52% cumulative on first three axes), which was primarily related to expansion and contraction of the posterior region of the fin.

Evaluation of Batoid Morphospace

Morphological disparity was four times larger in Myliobatoidei (disparity = 0.024) than Rajoidei (disparity = 0.0061), a distinction that was significant ($P < 0.0001$). However, when rays were split by morphotype, disparity was 0.0040 for pelagomorphs and 0.0039 for benthomorphs; the two were not significantly different ($P = 0.94$). However, disparity of rajoids was now significantly larger than that of benthomorphs ($P = 0.015$) and also larger than pelagomorph rays, but not significantly so ($P = 0.19$).

Procrustes ANOVA revealed that myliobatoids and rajoid pectoral shapes were not significantly different from each other (distance = 0.085, $P = 0.59$). However, when separated, the two myliobatoid morphotypes were significantly different (distance = 0.32, $P < 0.0001$), the benthomorphs were different than rajoids (distance = 0.14, $P < 0.0001$), and the pelagomorphs were also different from rajoids (distance = 0.22, $P < 0.0001$). Interestingly, these results suggest that skates are more similar in morphology to each of the ray morphotypes than
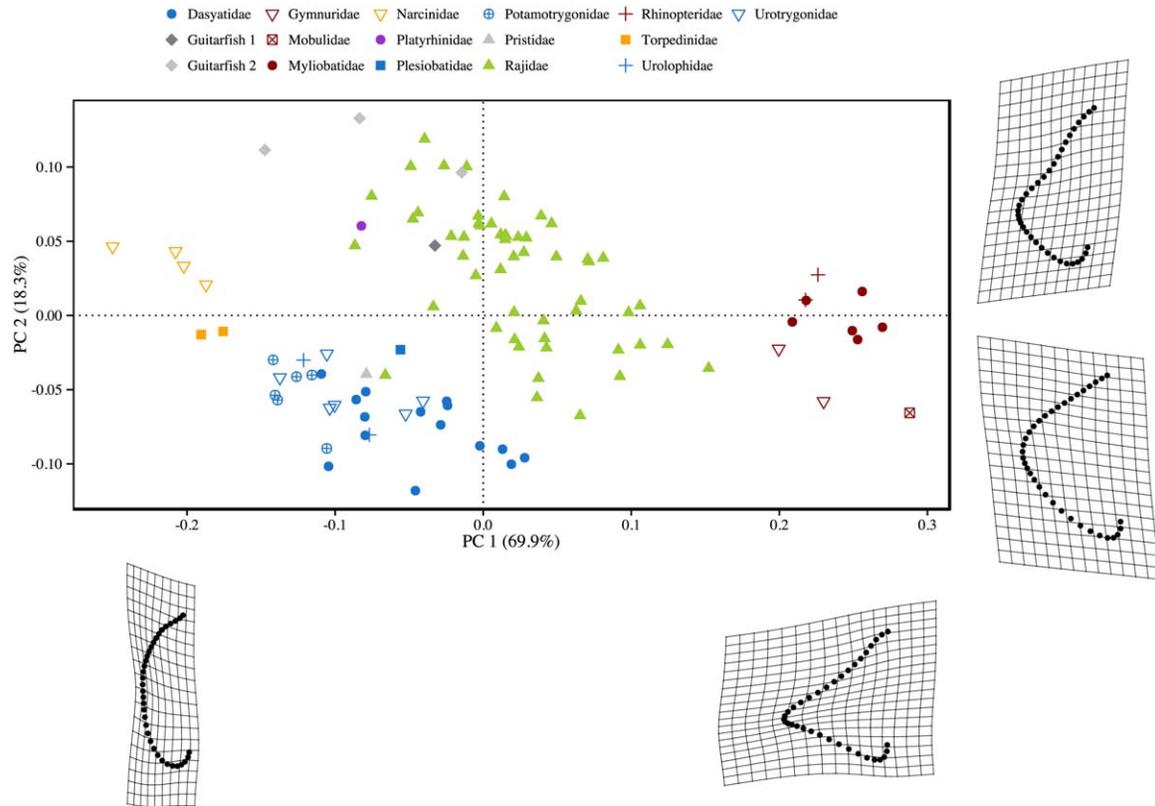
Fig. 4. Principal components (PCs) 1 and 2 for batoid pectoral shapes, with points colored by groups of interest in this study (blue = benthomorph myliobatoids, red = pelagomorph myliobatoids, green = rajoids, orange = torpedinoids, dark gray = guitarfish 1, light gray = guitarfish 2/pristid, purple = platyrhinoid). Shapes represent families within higher-level groupings. The percent of total shape variance explained by PCs is listed on respective axes. Shapes of PC endpoints relative to mean shape are provided as deformation grids.

the two are to each other. This latter set of comparisons, with separate myliobatoid morphotypes, is also more consistent with patterns of morphological variation visible on PCs 1 and 2 (Fig. 4).

In a PCA of pectoral shapes for just Myliobatoidei and Rajoidei, PCs 1 and 2 account for a majority (88.84%) of total shape variation (Supporting Information Fig. S2). Within this space, differences between benthomorph and pelagomorph myliobatoids set the upper and lower bounds of PC 1. Consequently, most of the shape change for myliobatoids is aligned along PC 1 and has a very small and non-significant slope with respect to PC 2 (slope = -0.036, $P = 0.23$). However, when myliobatoids are grouped by morphotype, it becomes apparent that benthomorph rays also have significant variation on PC2 (slope = 0.32, $P = 0.0003$) that is masked when considered jointly with pelagomorphs. Likewise, rajoid shape change on PCs 1 and 2 has a positive and significant slope (0.49, $P < 0.0001$).

Separate PCAs for rajoids and benthomorph myliobatoids provide further evidence that shape changes in the two groups are similar in nature (Fig. 5). In both, the primary axis of shape change is associated with lateral expansion and contraction of the fin apex and the secondary with flattened versus rounded anterior pectoral fins (refer to Supporting Information Fig. S3 and S4 for distributions of genera within each group).

Functional Implications of Pectoral Shape

Regressions of shape on aspect ratio (AR) were significant for all batoids and also for the pectoral-fin-based swimmers (Myliobatoidei plus Rajoidei), although the strength of the relationship (R^2) was just slightly greater in the latter (Table 1A). Tests of slopes between myliobatoids and rajoids revealed highly significant differences in the distance between slopes (distance = 0.101, $P < 0.0001$) and also in the angle between slopes ($\text{angle}_{\text{degr}} = 39.23$, $P < 0.0001$). In comparison, slopes of benthomorph myliobatoids and rajoids were more similar; slope distance between groups was not significant (distance = 0.059, $P = 0.058$), but the angle was ($\text{angle}_{\text{degr}} = 20.91$, $P = 0.035$) despite being approximately half as large as the angular difference between suborders. Regressions of PC 1 on AR were also significant and had an even larger R^2 than regressions made with full shape data (Table 1B, Supporting Information Fig. S5). Other axes, however, had much weaker and nonsignificant relationships with AR (Supporting Information Tables S2 and S3). Also, PGLS
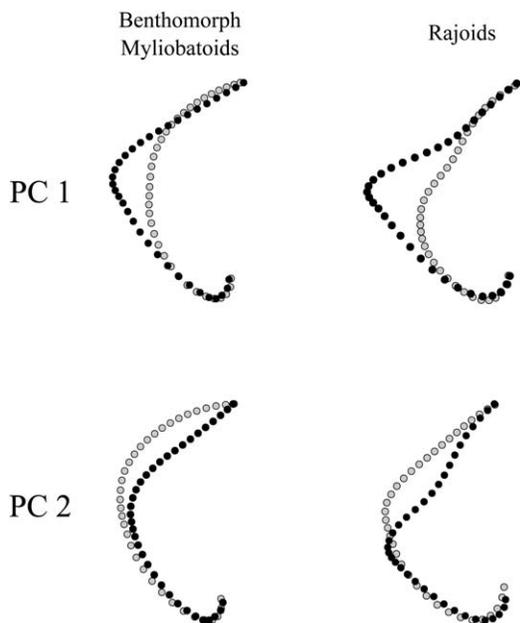
Fig. 5. Shapes of PC extremes for separate PCAs of benthomorph myliobatoids and rajoids. Minimum and maximum shapes superimposed to emphasis changes on given axes.

TABLE 2. Results from phylogenetic generalized least squares regressions (PGLS) of (A) shape versus aspect ratio (AR) and (B) PC 1 versus AR

	n	SS	df	R^2	F	P-value
A. shape versus AR						
all batoids	26	5.1003	1	0.60354	36.54	<0.0001
Myliobatoidei & Rajoidei	19	4.8173	1	0.70042	39.75	<0.0001
B. PC 1 versus AR						
all batoids	26	5.2192	1	0.85209	138.27	<0.0001
Myliobatoidei & Rajoidei	19	5.4235	1	0.78855	63.40	<0.0001

PC 2 scores and wave number showed no relationship ($r = -0.084$, df = 7, $P = 0.830$).

Rates of Shape Evolution

The rate of pectoral fin shape evolution was nearly three times faster in Myliobatoidei ($\sigma^2 = 0.00648$) compared to Rajoidei ($\sigma^2 = 0.00225$) and the difference between the two was significant ($P < 0.0001$). When divided by morphotype, the rate of evolution in pelagomorphs ($\sigma^2 = 0.0117$) was three times faster than benthomorph myliobatoids ($\sigma^2 = 0.0359$) and over five times faster than the aforementioned rate in rajoids. Pairwise comparisons of myliobatoid morphotypes and rajoids were all significant ($P < 0.0001$). An additional comparison of habitats, independent of suborder and morphotype designations, resulted in fully pelagic species with a rate of $\sigma^2 = 0.00334$, versus $\sigma^2 = 0.0118$ in benthic/demersal species. Therefore, the rate of change in pelagic species was approximately three times that of benthic and demersal species ($P < 0.0001$).

Like pectoral shape, the myliobatoid AR rate ($\sigma^2 = 19.64$) was significantly higher ($P < 0.0001$) than in rajoids ($\sigma^2 = 1.507$). The rate in pelagomorph myliobatoids was larger ($\sigma^2 = 40.12$) than that of benthomorphs ($\sigma^2 = 8.266$), which was also larger than that of rajoids; all pairwise comparisons were significant ($P < 0.0001$). Last, the fully pelagic species had a rate of AR evolution ($\sigma^2 = 46.69$) that was nearly ten times that of benthic/demersal species ($\sigma^2 = 4.680$) and again was significant ($P < 0.0001$).

Homology of Pectoral Fin Landmarks

The inclusion of a landmark at the anterior-most point of the batoid disc created a substantial source of added variation. Although the first PC described shape differences similar to our original configuration, the amount of variation explained on this axis decreased to 41.6% (28.3% less than before). This occurred as a consequence of added variance on PC 2 (36.6%), which was largely dominated by location of the anterior landmark (Fig 3 and Supporting Information Fig. S6).
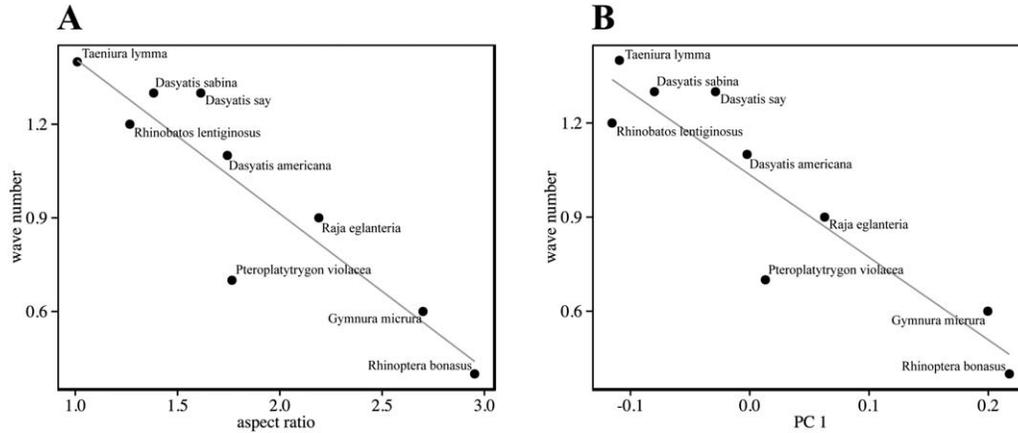
regressions of shape data and PC 1 on AR were all significant and generally consistent with results using the complete, nontransformed dataset (Table 2). Ancestral state reconstructions of AR (Supporting Information Table S4) were consistent with our understanding of batoid pectoral diversity, namely that axial-based swimmers had low AR, rajoids displayed continuous variation centered in low to intermediate AR and myliobatoids were highly bimodal. An interesting result was that the ancestor of Myliobatoids had an AR that was intermediate relative to all extant species. We do, however, suggest caution when interpreting these ancestral estimates, as additional taxon sampling may reveal other patterns (e.g., inclusion of *Hexatrygon*, which is sister to all remaining myliobatoids and has a unique morphology with fairly rounded fins).

Pectoral wave number (i.e., swimming mode) was negatively correlated with PC 1 scores ($r = -0.91$, df = 7, $P = 0.00062$) and aspect ratio ($r = -0.91$, df = 7, $P = 0.00064$; Fig. 6). In comparison,

TABLE 1. Regression results from comparisons of (A) shape versus aspect ratio (AR) and (B) PC 1 versus AR

	N	SS	df	R^2	F	P-value
A. shape versus AR						
all batoids	106	1.1751	1	0.6222	171.28	<0.0001
Myliobatoidei & Rajoidei	96	0.9462	1	0.6534	173.40	<0.0001
B. PC 1 versus AR						
all batoids	106	1.17199	1	0.88724	818.28	<0.0001
Myliobatoidei & Rajoidei	96	0.94481	1	0.9329	1278.40	<0.0001

Fig. 6. Pectoral wave numbers from Rosenberger (2001) plotted against (**A**) aspect ratios and (**B**) PC 1 scores.

Pectoral radials in Myliobatoidei extend to the midline of the body, causing the additional landmark to be placed approximately adjacent to the most anterior pectoral fin landmark (Fig. 1A). Consequently, the anterior landmark did not alter overall shape in this suborder as much as it did for others (Supporting Information Fig. S7). For example, some rajoids have pectoral fins that extend close to their rostral tips (e.g., genus *Bathyraja*), making them appear artificially closer in shape to the benthic ray morphotypes. The most notable impact of the additional landmark, however, occurred in the guitarfishes, Pristidae, and Torpedinoidei (Supporting Information Fig. S6), where the anterior point of the pectoral fin fell well short of the anterior of the disc (Fig. 1C & D). The anterior landmark also reduced the pectoral shape variance explained by regression on aspect ratio. Regression of Procrustes coordinates on AR for all batoids was still significant ($P < 0.0001$) but the relationship was weaker than it was previously ($R^2 = 0.376$, compared to 0.622 for the original shape configuration).

DISCUSSION
Overview of Batoid Pectoral Diversity

Our analysis of batoid pectoral morphology confirmed a well-established pattern of variation across the group, but also revealed a number of novel aspects of diversity within and among major clades. As in previous studies by Fontanella et al. (2013) and Franklin et al. (2014), we recovered a primary direction of shape change in Batoidea relating to, at one extreme, rounded body forms and at the other, highly angled bodies (PC 1, Fig. 4). Within Myliobatoidei, shapes occurred in one of two clearly separated morphological clusters, differentiated primarily by degree of lateral expansion, which we termed pelagomorphs (laterally expanded, highly angular) and benthomorphs (laterally contracted,

rounded). While it is apparent that the total range of pectoral forms in Myliobatoidei is the largest among batoid suborders, their bimodal distribution of shapes make direct comparisons of diversity difficult relative to other continuously distributed clades, like Rajoidei. One consequence of bimodality is that morphological disparity of myliobatoids is directly related to the size of the gap between the two morphotypes, where no ray shapes actually exist. Therefore, a clade with a bimodal shape distribution and large separation between morphological groups (black dots in Fig. 7) can hypothetically have greater shape disparity while occupying a much smaller portion of morphospace than a unimodal clade with larger variation of forms (white dots in Fig. 7). This partially explains why shape disparity of myliobatoids was so much larger than that of rajoids, while rajoids had a larger morphological disparity than each of the myliobatoid morphotypes. Overall, however, myliobatoids still appear to have larger morphological variance than rajoids, but the difference (in terms of variety of forms) is not as great as it initially appears in direct comparisons of suborders.

Another noteworthy outcome was that several rajoid species occupied the aforementioned gap between myliobatoid morphotypes (Fig. 4), therefore, having intermediate aspect ratios relative to benthomorph and pelagomorph rays (Supporting Information Fig. S5). While this has important implications for relative diversity of function and lifestyle in skates versus rays, it causes another analytical difficulty. When a clade has bimodal shape variation, as in the myliobatoids, mean shape may be outside of the individual distributions of either morphotype. Furthermore, the existence of another clade in the gap between morphotypes means that both clades may have similar mean shapes but little or no overlapping forms (Fig. 7). This explains why rajoids and myliobatoids were not significantly different under Procrustes ANOVA, but rajoids were significantly
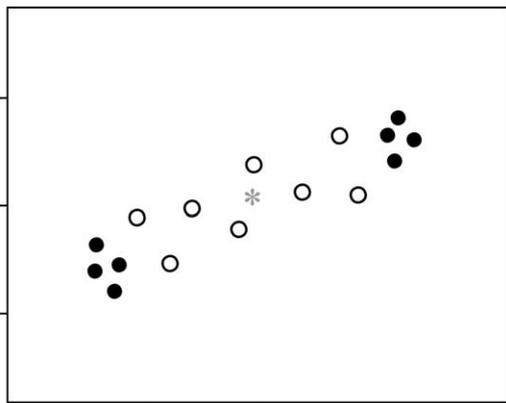
Fig. 7. Hypothetical two-dimensional morphospace displaying shape variation in two clades, one with a bimodal shape distribution (black dots) and the other with a continuous distribution (white dots). The approximate mean shape for both clades is marked by an asterisk.

different from each myliobatoid morphotype and were visually separated from them on a PCA plot of tangent shape space (Fig. 4). The location of skates in batoid morphospace also meant that they were more similar in overall morphology to each ray morphotype than the two morphotypes were to each other.

One of the more prominent features of batoid pectoral diversity was the similar and nearly parallel shape trajectories of rajoids and benthomorph myliobatoids (Fig. 5 and Supporting Information Fig. S2). A pair of morphological transformations defines shape changes common to both groups; in addition to lateral expansion/contraction of the pectoral apex, both the rajoids and benthomorph myliobatoids varied in the overall roundness or curvature of their fins (PC 2, Fig. 5). There was no indication that shape variation occurring on PC 2 impacted AR (Supporting Information Table S3). Although roughly parallel, skates were shifted in morphospace relative to the benthomorph rays, such that skates had larger lateral expansion but the anterior half of fins in benthomorphs achieved a stronger convex curvature (Figs. 4 and 5).

Implications for Locomotion and Lifestyle

The anteroposterior expansion of batoid pectoral fins has resulted in overall body plans ranging from shark-like (e.g., pristids) to nearly circular (e.g., potamotrygonids). As a whole, however, arguably no other group of fishes has featured the pectoral fin in such a prominent way. Regression results highlighted the degree to which batoid pectoral shape translates to variation in AR, a functional trait that is known to influence lift and thrust generation (Daniel, 1988). PC 1 scores and AR had a particularly strong relationship (Tables 1B and 2B, Supporting Information Fig. S5). However, assigning traits to PCs (here AR) and inter-

preting them as such can be a dubious exercise, as the axes are simply constructs used to display low dimensional views of higher dimensional data and have no inherent biological basis. Our use of PC 1 in this context is primarily to convey that a considerable component of pectoral shape variation in batoids is associated with AR, and therefore, function. Additionally, both PC 1 and AR were strongly and similarly related to pectoral wave number (Fig. 6), which is the determinant of swimming mode along the undulation-oscillation continuum.

The above relationships provide evidence that pectoral shape variation is a critical component for the evolution of swimming modes in batoid fishes and are consistent with the results of recent studies on batoid morphology (Fontanella et al., 2013; Franklin et al., 2014). In particular, Fontanella et al. (2013) showed that aspect ratio was related to swimming mode, which they coded as oscillatory or undulatory. While a categorical treatment of swimming mode may be useful for demonstrating general patterns, the nature of observed swimming diversity (Rosenberger, 2001). and distribution of aspect ratios across batoids (Supporting Information Fig. S5) suggest that a continuous representation may be more appropriate. Rosenberger's kinematic study formed the basis of swimming-related comparisons in this research (Fig. 6) and also in the two recent batoid morphology manuscripts. However, in that initial study, swimming mode was assessed primarily in myliobatoids, with only one representative guitarfish and one rajoid. If relationships between pectoral shape and swimming mode (Fig. 6) hold true across the rajoids, then it suggests that we have been underestimating the swimming and lifestyle diversity contained within this clade. Using myliobatoid diversity as a relative indicator, rajoids potentially vary from undulatory (species overlapping with benthomorph rays) to near-pelagic levels of oscillation (species occupying intermediate shape and AR between ray morphotypes). Although rajoids can be quite mobile and undergo seasonal migrations (Frisk, 2010), the range of swimming and lifestyle diversity implied in this research is beyond what is currently known for skates, and a reappraisal of their functional and ecological diversity may be warranted.

The observed bimodality in myliobatoids is consistent with different functional demands associated with benthic environments versus pelagic. However, substantial variation solely within the benthomorphs point to significant diversity in locomotion and lifestyle within this group alone. Species with low AR and rounded bodies, like the potamotrygonids, have pectoral fins suited for fine-scale maneuverability within their riverine environments (for a discussion on turning and maneuverability in batoids, see Parson et al., 2011). At the other extreme were several dasyatids with rhomboid-shaped fins and larger AR that live more vagile lifestyles, some

undergoing seasonal migrations, like *Dasyatis centroura* (Struhsaker, 1969). A similar direction of variation in rajoids hints that shape variation may also be linked to activity level.

Maximum likelihood state reconstructions of AR, albeit on a reduced set of taxa, resulted in the ancestor to Rajoidei having a low to intermediate AR of 1.84 ± 0.40 SD and the ancestor to Myliobatoidei with an intermediate AR of 2.10 ± 0.27 SD (Fig. 2, Supporting Information Table S4). Although these estimates are not wildly different from each other, the distributions of AR in extant taxa tell the story of two very different processes resulting in the current distributions of pectoral shapes in rajoids and myliobatoids. Aspect ratio varies continuously in rajoids, with the ancestral state nested within extant forms. In contrast, the AR estimate for the ancestral myliobatoid state, if accurate, was intermediate between all extant ray species and actually falls within the range of current rajoids (Supporting Information Fig. S5). The larger morphological and functional transition from ancestral to extant states exhibited in myliobatoids may, therefore, explain the more rapid evolutionary rates of shape and AR change relative to rajoids. In addition, rate estimates solely based on habitat showed that pectoral fins of pelagic species evolved much more rapidly than benthic and demersal species, which makes sense based on the relatively large functional jump required for a fully pelagic lifestyle.

Potential Morphological Constraint in Rajoids

Despite the proximity of some rajoids to the morphospace occupied by pelagomorph myliobatoids and also having aspect ratios that are not extremely distant from pelagic species, it is interesting that there are no fully pelagic skates. It is possible that skates are morphologically constrained and that the adaptive peak for a fully pelagic lifestyle is not attainable with the rajoid body plan. One of the shared traits in pelagic rays is that the anterior fin margins are convex while the posterior margins are concave, giving the pectoral fin an overall crescent shape. This shape is found in several aquatic organisms as well as some birds and is believed to increase efficiency of oscillatory locomotion (van Dam, 1987). Even the pelagic stingray (*P. violacea*) with its benthomorph body style, has a convex anterior fin with a very flat and even slightly concave posterior margin in large adults. In contrast, the rajoids all have fins in which the posterior half is convex and also particularly enlarged and rounded near its base. Additionally, the anterior half of the rajoid fin ranges from concave to slightly convex, but never to the extent seen in myliobatoids (PC 2 in Fig. 5). Therefore, an inability or lack of opportunity for rajoids to attain the crescent-shaped fin of

pelagic species may have precluded them from fully pelagic lifestyles. Another interesting contrast is observed in the myliobatoid family Gymnuridae, where species are superficially similar to pelagic rays with large AR, but differ in having a convex posterior fin. Rosenberger (2001) notes that the species *Gymnura micrura* can shift between more undulatory and oscillatory swimming when in benthic and pelagic environments, giving them unique and essentially hybrid swimming capabilities. This suggests that while high AR can help to achieve oscillatory swimming, a pelagic lifestyle in batoids may be predicated on a crescent-shaped pectoral fin with a convex leading edge and concave trailing edge.

Additional Factors Influencing Locomotion

The example of the pelagic stingray, *P. violacea*, demonstrates that pectoral shape is not the sole factor determining locomotion and ultimately lifestyle in batoids. One important element impacting fin function is its rigidity. In batoids, several structural modifications impact stiffness of pectoral fins, including mode of calcification, relative spacing and staggering of radial joints, as well as cross-bracing of adjacent radials (Schaefer and Summers, 2005). Additionally, Fontanella et al. (2013) showed that the distribution of mass within the fin differed between batoids with undulatory versus oscillatory swimming. The variation in cross-sectional symmetry influences water flow along the fin and results in hydrodynamic effects that are suited to different habitats used by each. Behavior is yet another way that batoids may control their swimming. For example, the guitarfish *Rhinobatos lentiginosus* often swims with its body angled upward from the horizontal (Rosenberger, 2001). In addition, many batoids can alter properties of pectoral waves (e.g., frequency, wavespeed, and wave number), depending on flow conditions (Rosenberger, 2001). Finally, several batoid species use pelvic fins to supplement locomotion for specific tasks like benthic walking and propelling themselves into the water column, termed punting (Koester and Spirito, 2003). The shape of the pelvic girdle has even been suggested as an indicator of overall swimming mode (Ekstrom and Kajiura, 2014).

Comments on Pectoral Homology and Landmark Use

In geometric morphometrics, landmark placement is vital to the efficacy of analyses (Zelditch et al., 2004) and can be particularly challenging for outlines as they often have few distinguishable homologous points, if any (Gunz and Mitteroecker, 2013). Both this study and Franklin et al. (2014) used geometric morphometrics to capture shape variation in pectoral fin outlines across wide ranges of batoid taxa. Both studies recovered a primary

direction of pectoral variation associated with low versus high aspect ratio fins, but also differed in key aspects that resulted in dissimilar relationships within and among the major batoid lineages (compare Fig. 4 to Fig. 3 in Franklin et al., 2014). It appears that at least some of the differences between studies can be explained by landmark choices and treatments of shape data.

As outlined in the introduction, homology of the pectoral fin is defined by specific anatomical structures that are shared in all elasmobranchs. In Franklin et al. (2014), the anterior extent of pectoral fins were consistently defined at the anterior-most point of the batoid disc (Fig. 1). However, in a majority of batoids pectoral fins do not extend to this location, and in some cases the distinction is quite large. This landmark treatment introduced a bias whose magnitude depends on the taxa considered (Supporting Information Fig. S7); the addition of anterior nonpectoral points on the rostra of pristids and guitarfishes caused the lateral apex of the fin to appear posterior to its actual location on the fin (a dominant feature of PC 2 in Fig. 3 of Franklin et al., 2014) and anterior landmarks over part of the enlarged electric organs of torpedinoids (Fig. 1) made them appear artificially more similar to rajoids and benthic myliobatoids. In fact, when we added an additional anterior point to our shape configurations, we were able to generate a morphospace where PC 2 was similarly driven by the displacement of the fin apex relative to the anterior disc margin, as in Figure 3 of Franklin et al. (2014). Another matter of homology was the placement of the posterior endpoint and the fixed lateral apex point in Franklin et al. (2014), both of which are defined as points of tangency on the pectoral fin. Landmarks of this nature are consistent with type 3 landmarks or extremal points, described by Bookstein (1991), and their effectiveness in representing homologous features has been questioned. In batoids, placement of these points in a rounded species would be much more difficult than in a species with sharply angled fins.

Another difference between our results and that of Franklin et al. (2014) was in the diversity of Rajoidei. Their work did not suggest the same level and type of rajoid shape variation as the current study. We believe this to be at least partially because the authors averaged shapes at the genus level and not by species, as done here. This may disproportionately average away shape variation in rajoids due to their very high species diversity compared to other batoids (especially genera like *Bathyraja* or *Raja* that display large shape variation, Supporting Information Fig. S3). It was also unexpected that in the previous study, rajoids did not appear to display large variance in aspect ratio (i.e., PC 1). Although Franklin et al. (2014) did not include some of the rajoid genera that varied most in aspect ratio in our study (e.g., low AR: *Brevir-*

aja, *Dactylobatus*, *Fenestraja*; high AR: *Rostroraja*, *Zearaja*; Supporting Information Fig. S3), they did use species as wide ranging in shape as *Psammobatis lentiginosa* and *Atlantoraja cyclophora* (Supporting Information Fig. S8), which would be expected to hold more divergent positions on PC 1 (related to lateral expansion of the fin) than they did in their study.

CONCLUSIONS

Batoids have achieved surprising morphological diversity with a relatively simple body plan. The observed distribution of morphologies in these fishes displays clear directionality, and grouping of species follows expectations based on locomotion and lifestyle. This work has provided new insight on patterns of diversity among batoid lineages and we hope that it motivates further questions about the mechanisms and drivers of evolution in this group. For example, a similarly broad study comparing the structural design of endoskeletons in different batoid taxa is certainly warranted and may provide understanding on potential morphological constraints in rajoids that prevent fully pelagic lifestyles. Another area for future work is the influence of size on the shapes attained in different batoid clades. This may require relying less on archival collection materials, as size in large species is often underrepresented due to space limitations.

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