

# The evolution of underwater flight: The redistribution of pectoral fin rays, in manta rays and their relatives (Myliobatidae)

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## Abstract

Batoids are a diverse clade of flat cartilaginous fishes that occur primarily in benthic marine habitats. The skates and rays typically use their flexible pectoral fins for feeding and propulsion via undulatory swimming. However, two groups of rays have adopted a pelagic or benthopelagic lifestyle and utilize oscillatory swimming—the Myliobatidae and Gymnuridae. The myliobatids have evolved cephalic lobes, anteriorly extended appendages that are optimized for feeding, while their pectoral fins exhibit several modifications that likely arose in association with functional optimization of pelagic cruising via oscillatory flight. Here, we examine variation in fin ray distribution and ontogenetic timing of fin ray development in batoid pectoral fins in an evolutionary context using the following methods: radiography, computed tomography, dissections, and cleared and stained specimens. We propose an index for characterizing variation in the distribution of pectoral fin rays. While undulatory swimmers exhibit symmetry or slight anterior bias, we found a posterior shift in the distribution of fin rays that arose in two distinct lineages in association with oscillatory swimming. Undulatory and oscillatory swimmers occupy nonoverlapping morphospace with respect to fin ray distribution illustrating significant remodeling of pectoral fins in oscillatory swimmers. Further, we describe a derived skeletal feature in anterior pectoral fins of the Myliobatidae that is likely associated with optimization of oscillatory swimming. By examining the distribution of fin rays with clearly defined articulation points, we were able to infer evolutionary trends and body plan remodeling associated with invasion of the pelagic environment. Finally, we found that the number and distribution of fin rays is set early in development in the little skate, round stingray, and cownose ray, suggesting that fin ray counts from specimens after birth or hatching are representative of adults and therefore comparable among species.

## KEY WORDS

batoid, cephalic lobe, functional optimization, oscillatory, undulatory

## 1 | INTRODUCTION

Batoids are a diverse group of cartilaginous fishes, with a body plan adapted to benthic life. The group is composed of the skates, thornbacks, electric rays, guitarfishes, sawfishes, and stingrays, and together form a clade that is sister to sharks (Aschliman et al., 2012). Batoids exhibit significant modifications to their body plan relative to sharks including dorso-ventral compression with pectoral fins that extend anteriorly and fuse to the head. While vertebrates exhibit left-right bilateral symmetry in the skeleton (Romer, 1950), we have observed

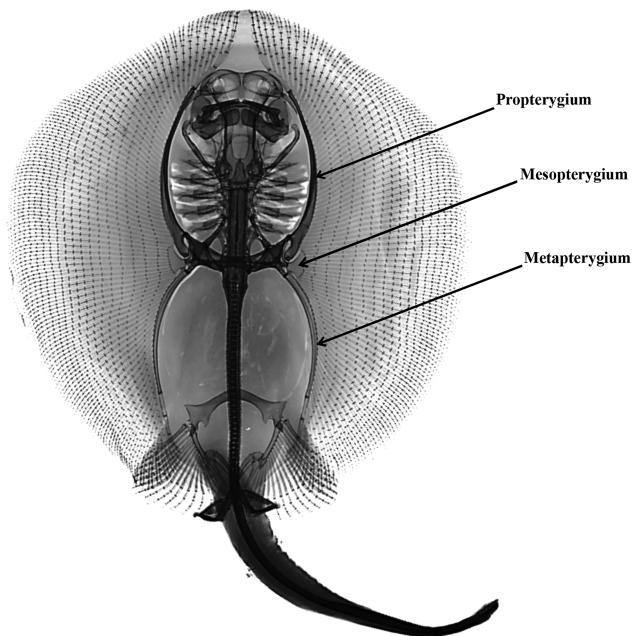
that some skates and rays have an additional line of symmetry dividing the pectoral fin rays into anterior and posterior domains, and this secondary axis of symmetry may have functional significance.

While the thornbacks and guitarfishes primarily use caudal fin propulsion for axial swimming, the skates and rays utilize their pectoral fins for locomotion (Breder, 1926; Klausewitz, 1965; Rosenberger, 2001). In most skates and rays, the pectoral fins are used for both undulatory swimming and feeding (Mulvany & Motta, 2014). Undulatory swimming, in which multiple waves are simultaneously propagated along the pectoral fin margin, is a maneuverable gait that is

mechanically efficient for hovering and swimming above the benthos at low speeds, but not for travelling long distances (Di Santo, Blevins, & Lauder, 2017; Rosenberger, 2001; Walker & Westneat, 2000; Webb, 1994). Undulatory pectoral fins are used in feeding to constrain prey items under the body by pectoral fin edges pressed against the substrate in a behavior called "tenting" (Wilga, Maia, Nauwelaerts, & Lauder, 2012; Wilga & Motta, 1998). This versatility suggests that undulatory pectoral fins are optimized for flexibility and multifunctionality.

Species from two families including the Myliobatidae (manta and devil rays, cownose rays, bat rays and eagle rays) and Gymnuridae (butterfly rays) have adopted a more pelagic lifestyle and utilize the oscillatory swimming mode. As such, they have several derived pectoral fin modifications, including lateral expansion of the pectoral fins resulting in an increased aspect ratio relative to the ancestral batoid body plan (Fontanella et al., 2013), anteroposterior elongation of the pectoral girdle (scapulocoracoid, Da Silva & De Carvalho, 2015; McEachran, Dunn, & Miyake, 1996; Nishida, 1990), and stiffening by crural calcification of radials and cross-bracing among fin rays (Heine, 1992; Lighthill, 1969; Mulvany & Motta, 2013; Rosenberger, 2001; Schaefer & Summers, 2005). These derived morphologies are associated with oscillatory swimming, where the wing-like pectoral fins oscillate up and down in a flapping motion, analogous to flight in birds, and is characterized by having less than half a wavelength along the pectoral fin margin (Heine, 1992; Rosenberger, 2001; Webb, 1994). Relative to undulatory swimming, the oscillatory swimming mode minimizes drag while increasing both lift and thrust in a manner that is conducive to efficient cruising in the pelagic environment (Fish et al., 2016; Lighthill, 1969; Rosenberger, 2001; Webb, 1975).

Body plan modifications that stiffen the pectoral fins and facilitate pelagic cruising represent a trade-off in feeding efficiency. Gymnurids, which are capable of both undulatory and oscillatory swimming modes, utilize their pectoral fins to stun and pin prey to the substratum before ingesting it (Jacobsen, Johnson, & Bennett, 2009; Schreiber, 1997), thereby using their pectoral fins in a manner reminiscent of the "tenting" mechanism described in undulatory skates and rays. Alternatively, the myliobatids utilize cephalic lobes, anteriorly extended appendages used exclusively for feeding (Mulvany & Motta, 2014), which likely released evolutionary constraints on the pectoral fins, resulting in increased evolvability (see Kirschner & Gerhart, 1998) and optimization for locomotor behaviors. Cephalic lobes exhibit morphological variation ranging from a single, fused broad cephalic lobe in the Myliobatinae (bat rays and eagle rays), paired cephalic lobes in the Rhinopterinae (cownose rays) that come together at the midline in front of the mouth, and the anteriorly extended yet separate cephalic lobes in the Mobulinae (devil rays and manta rays, McEachran et al., 1996, and see Nelson, 2006 for subfamily designations) (see fig. 1 illustration in Mulvany & Motta, 2013). Cephalic lobes are used in feeding in a variety of ways depending on lifestyle and morphology. The Myliobatinae and Rhinopterinae both have benthic-pelagic lifestyles that include feeding on benthic prey, which are detected by electrosensory pores distributed on the ventral side of the cephalic lobes (Mulvany & Motta, 2013, 2014; Sasko, Dean, Motta, & Hueter, 2006). The Myliobatinae use their



**FIGURE 1** Skeletal structure. Representative batoid (*Urotrygon maculatus*) to display the three primary cartilages: Propterygium, Mesopterygium, and Metapterygium which support the pectoral fin

broad fused cephalic lobe to dig in the benthos to uncover benthic invertebrates (Gudger, 1914; Michael, 2005), while the Rhinopterinae use their split cephalic lobes primarily to trap prey against the substrate (Mulvany & Motta, 2013; Sasko et al., 2006). The Mobulinae solely inhabit the pelagic environment and utilize their large flexible cephalic lobes to direct plankton into their mouths during filter feeding (Motta & Huber, 2004; Notarbartolo-di-Sciara & Hillyer, 1989). As such, their head region has been remodeled to facilitate planktivory, and their wing-like cephalic lobes lack electrosensory pores (Mulvany & Motta, 2013).

Myliobatids exhibit separate domains that were independently optimized for feeding and oscillatory swimming (Mulvany & Motta, 2014). The mobulids, with their distinct cephalic lobes, have been described as the only living vertebrate to exhibit a third pair of functional appendages (Nelson, 2006). Other authors have described them as modified extensions of the anterior pectoral fins (Mulvany & Motta, 2013; Nishida, 1990). Cephalic lobes are supported by fin rays, as are pectoral and pelvic fins, but it is unknown whether the anterior pectoral fin rays of myliobatids have been repurposed as cephalic lobes, or if myliobatids have novel or additional anterior fin rays relative to taxa lacking cephalic lobes. Thus, our *a priori* hypothesis is that myliobatids exhibit an anteriorly biased distribution of fin rays, associated with the anteriorly expanded pectoral girdle and the presence of cephalic lobes (Da Silva & De Carvalho, 2015; McEachran et al., 1996; Nishida, 1990).

The aims of this study are fourfold: (a) quantify the number of fin rays with specific articulation points in batoid pectoral fins; (b) determine when in ontogeny the number of pectoral fin ray elements is set; (c) develop an index for characterizing variation in fin ray distribution; and (d) evaluate that variation in a phylogenetic context to examine trends associated with the evolution of distinct swimming modes and

separation of feeding and swimming functionality in the pectoral fins of the Myliobatidae.

## 2 | METHODS

### 2.1 | Fin ray visualization protocols

Fin ray counts from individuals were obtained by radiographed, scanned computed tomography (CT), dissected or cleared and stained specimens. The clear and stain protocol for *Leucoraja* and *Urotrygon* followed Gillis, Dahn, and Shubin (2009). We modified this protocol for myliobatids by incorporating steps for larger specimens following Summers (2015) implementing a 1.0% trypsin in 30% saturated sodium borate solution after staining in alcian blue followed by an EtOH rinse. Finally, after the KOH:glycerol graded series, a scalpel was used to remove thin layers of skin and muscle from medial areas of the pectoral fin on both the dorsal and ventral sides of each specimen in order to view fin ray articulation to the primary cartilage.

Dorsal-ventral radiographs were performed with a Cabinet X-ray System (Faxitron, Model 43855C) using Kodak Industrex MX-125, 14" × 17" Ready Pack film for specimens representing 12 species from nine genera housed in the ichthyology collection at the California Academy of Sciences. Voltage and exposure times for radiographs varied depending on the specimen thickness. We also obtained fin ray counts from a radiograph collection at the University of Washington (Adam Summers "APS" personal collection) representing 11 species, and from seven species of cleared and stained specimens. Specimens were photographed using a Canon 5D Mark III with a 100 mm macro lens and MP-E 65mm f/2.8 1–5× macro lens for larger and smaller specimens, respectively. All images in Figures 1–8, Supporting Information Figures S1, S3, and S4 of radiographed or cleared and stained specimens were optimized in Adobe Photoshop CS6 (Adobe Systems, San Jose, CA). Additional radiographs were obtained via open access websites: five species from the California Academy of Sciences, three species from the Atlas of Living Australia and four species from the Smithsonian National Museum of Natural History. CT-scans of four species of rays were obtained from Sharksandrays.org. Details regarding visualization methods, sample size, and source of specimens are included in Table 1.

### 2.2 | Pectoral fin ray counts and articulation points

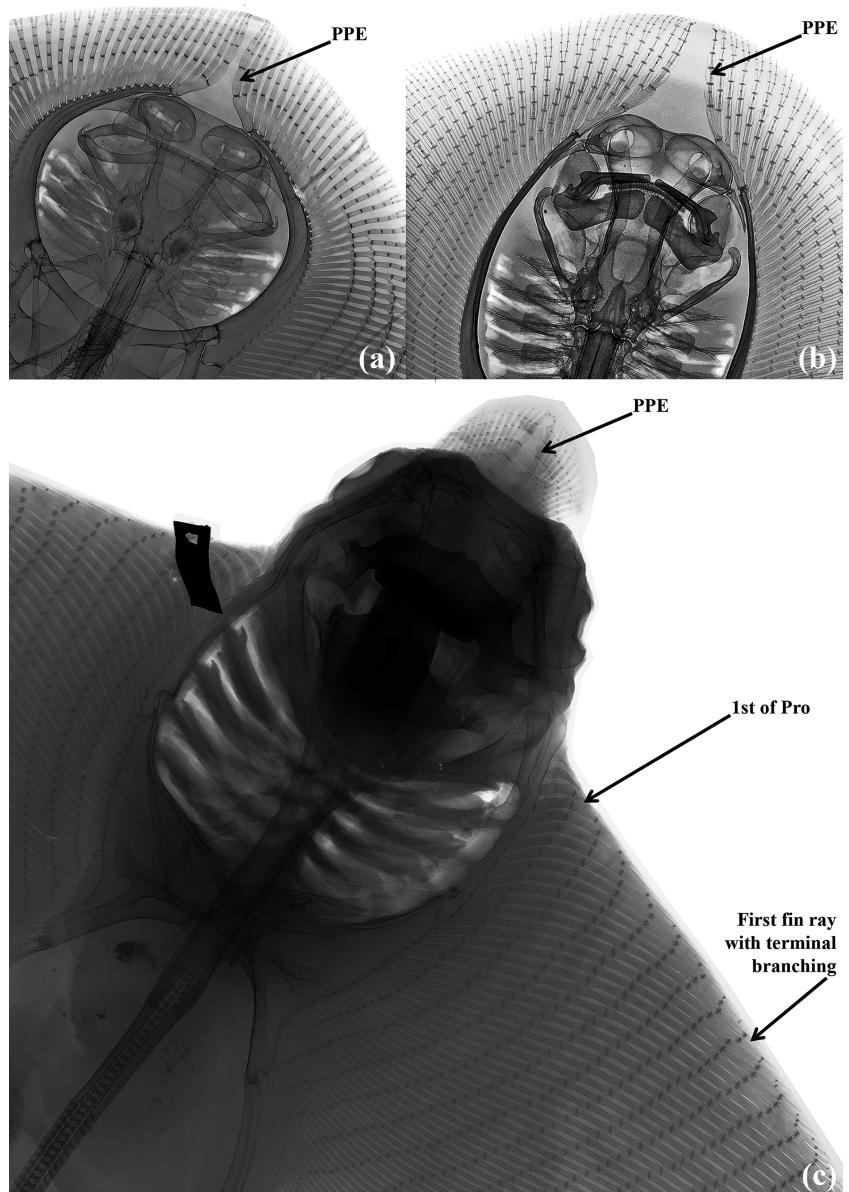
Skeletal support in pectoral fins of cartilaginous fishes are composed of the scapulocoracoid (pectoral girdle) and three primary cartilages (the propterygium, mesopterygium, and metapterygium) which span the anterior, middle, and posterior pectoral fin, respectively (Figure 1). In most batoids, the scapulocoracoid has three horizontally arranged condyles along the lateral aspect, one each for the propterygium, mesopterygium, and metapterygium (McEachran et al., 1996). However, several taxa, such as *Aetobatus*, *Rhinoptera*, and *Mobula*, lack or exhibit a fused mesopterygium (Garman, 1913; Lovejoy, 1996), while *Myliobatis* and *Gymnura* exhibit a fragmented mesopterygium, with some elements articulating with the scapulocoracoid (Nishida, 1990). Therefore,

we considered fin rays in the middle of the pectoral fin to be associated with the "mesopterygium" if they did not distinctly articulate with the propterygium or metapterygium.

Criteria for meristic counts of batoid pectoral fin rays vary in the literature. Some studies count all fin rays with terminal branching, including fin rays that extend the propterygial and metapterygial axes (Compagno & Roberts, 1982), while others exclude the anterior and posterior distal extensions of the primary cartilages (Last, White, & Pogonoski, 2008). Further, the number of segments in the anterior and posterior primary cartilages is variable across batoids (Da Silva & De Carvalho, 2015; Lovejoy, 1996; Nishida, 1990; Rosenberger & Westneat, 1999). Therefore, we clarify articulation points and characteristics of fin rays included in meristic counts, independent of segment number in primary cartilages, so that future studies on pectoral fin ray meristics can be consistent. In this study, we refer to the anterior and posterior extensions of the primary cartilages, as the principal propterygial elements (PPE) in the anterior, and the terminal metapterygial elements (TME) in the posterior. Fin rays articulate with the primary cartilages and are composed of multiple elements called radials which are separated by inter-radial joints (Figure 1). Most fin rays exhibit terminal branching and articulate laterally from the primary cartilages toward the fin edge, and these were included in our meristic counts. However, some fin rays extend the axis of the primary cartilage, and the number varies within and between species. We did not count the first element in line with the propterygial axis that meets at the midline or rostrum. We called this collection of radials the PPE, which was not included in our counts even if it had terminal branching. However, in some species, the PPE was divided into one or two elements that lacked terminal branching, and these also were not included in our meristic counts. In these individuals, the first fin ray we counted exhibited terminal branching (Figure 2 and Supporting Information Figure S1). In the posterior primary cartilage, we defined the TME as the most posterior element for which the bifurcating fin rays articulate posteriorly (i.e., not laterally, Figure 3), in line with the primary cartilage axis, and these were not included in our counts. This is consistent with the fin ray counts in Last et al. (2008), who excluded fin rays articulating with the anterior propterygium and posterior metapterygium, because they were considered to be part of the main skeleton.

Most fin rays that we counted had terminal branching; however, there were multiple genera in the Myliobatidae with fin rays lacking terminal branching (located in the anterior pectoral fin). In these instances, we counted fin rays with one or more inter-radial joints. For example, there are small elements articulating with the propterygium in the anterior pectoral fin of some myliobatids that lack an inter-radial joint, and those were not included in our counts (Figure 2c).

Finally, we applied these same rules when counting fin rays in cephalic lobes of myliobatids. Counts were taken from both the left and right pectoral fins and cephalic lobes on specimens new to this study and compared with counts from previous studies (Table 1). When average fin ray counts were not provided (i.e., only the range was given) we chose the median, indicated by an asterisk next to the superscript source in Table 1.

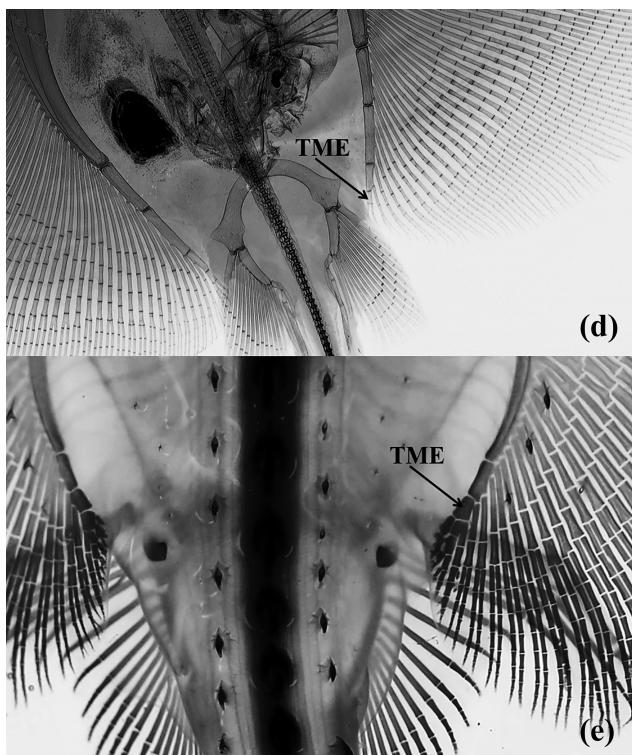


**FIGURE 2** Fin ray articulation points in Propterygium. Point of reference for PPE in varying genera and species of batoids. A: *Gymnura micrura* B: *Urotrygon maculata* C: *Aetobatus narinari*

### 2.3 | Number of fin rays throughout ontogeny

To evaluate whether the number of pectoral fin rays increases with allometric growth or is specified early in development, we quantified the number and distribution of fin rays in three batoid species. We evaluated samples that span the onset of pectoral fin development through hatching ( $n = 11$ ) and three adult specimens (Figure 4) in the egg laying taxon—the little skate (*Leucoraja erinacea*). Counts from cleared and stained embryos were obtained from University of Washington's Ichthyology collection at Friday Harbor Laboratories and we quantified fin ray counts from radiographs in three adult specimens obtained from the California Academy of Sciences. We also quantified the number of fin rays in round stingrays (*Urotrygon halleri*), which give live birth to average litters of two to three pups per brood (Ebert,

2003). Counts from cleared and stained embryos ( $n = 3$ ) were obtained from San Francisco State University's Vertebrate Museum Ichthyology collection we also quantified fin ray counts of one newborn, one juvenile, and three adult specimens from various sources (Table 1). Because myliobatids give birth to live young with extremely small broods, for example, single pups in *Rhinoptera* (Fisher, Call, & Grubbs, 2013) and *Mobula* (Notarbartolo-di-Sciara, 1988) and 2–12 in *Myliobatis californica* (number of offspring is dependent on size of mother, Herald, 1953; Martin & Cailliet, 1988), sampling opportunities of myliobatid pre-birth embryos is prohibitively difficult. However, we were able to obtain embryos ( $n = 3$ ) of the cownose ray (*Rhinoptera bonasus*) to compare with fin ray counts in adults ( $n = 6$ ), and a single embryo of the bat ray (*Myliobatis californica*) for comparison with three adults. We only



**FIGURE 3** Fin ray articulation points in Metapterygium. Point of reference for TME in varying genera and species of batoids. D: *Gymnura micrura* E: *Leucoraja erinacea*

included fin ray counts from embryos in which the propterygium was elongated and fused to the head/rostrum which corresponds to stage 32 in the winter skate (*Leucoraja ocellata*, Maxwell, Fröbisch, & Heppeleston, 2008), and is consistent with complete development of the visceral skeleton in the little skate (at approximately 80–100 mm total length, Gillis et al., 2009).

We compared the total number of fin rays in embryos with adults using the *wilcox.test* command (Wilcoxon rank sum test with continuity correction) in R (v.3.2.4, R Core Team, 2016).

## 2.4 | An index for characterizing variation in the distribution of fin rays

To illustrate evolutionary trends in the number and distribution of fin rays articulating with the anterior (propterygium) and posterior (metapterygium), we propose an index of fin ray distribution as

$$FR_{Dist} = \frac{FR_{Meta} - FR_{Pro}}{FR_{Tot}}$$

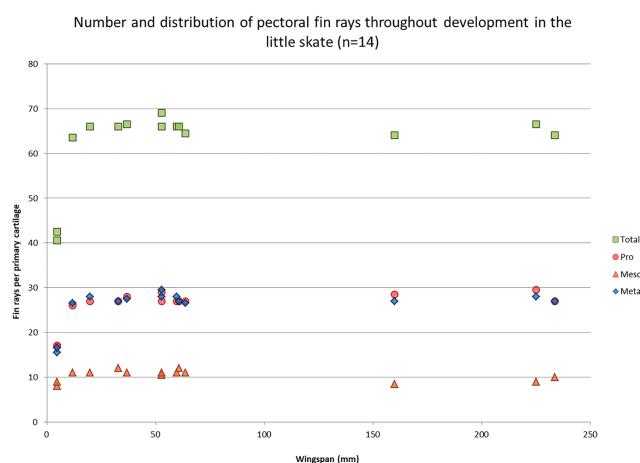
where  $FR_{Pro}$  is the number of rays articulating with the propterygium,  $FR_{Meta}$  is the number of rays articulating with the metapterygium, and  $FR_{Tot}$  is the total number of fin rays in the pectoral fin. When present, cephalic lobe fin rays are included as articulating with the propterygium. The theoretical boundaries of this index range from –1 (all fin rays distributed anteriorly and articulating with the propterygium) to 1 (all fin rays distributed posteriorly and articulating with the metapterygium), while a value of zero represents anterior-posterior (AP) symmetry, with equal numbers articulating with the propterygium and

metapterygium. We note that the range of values that are biologically relevant is much smaller.

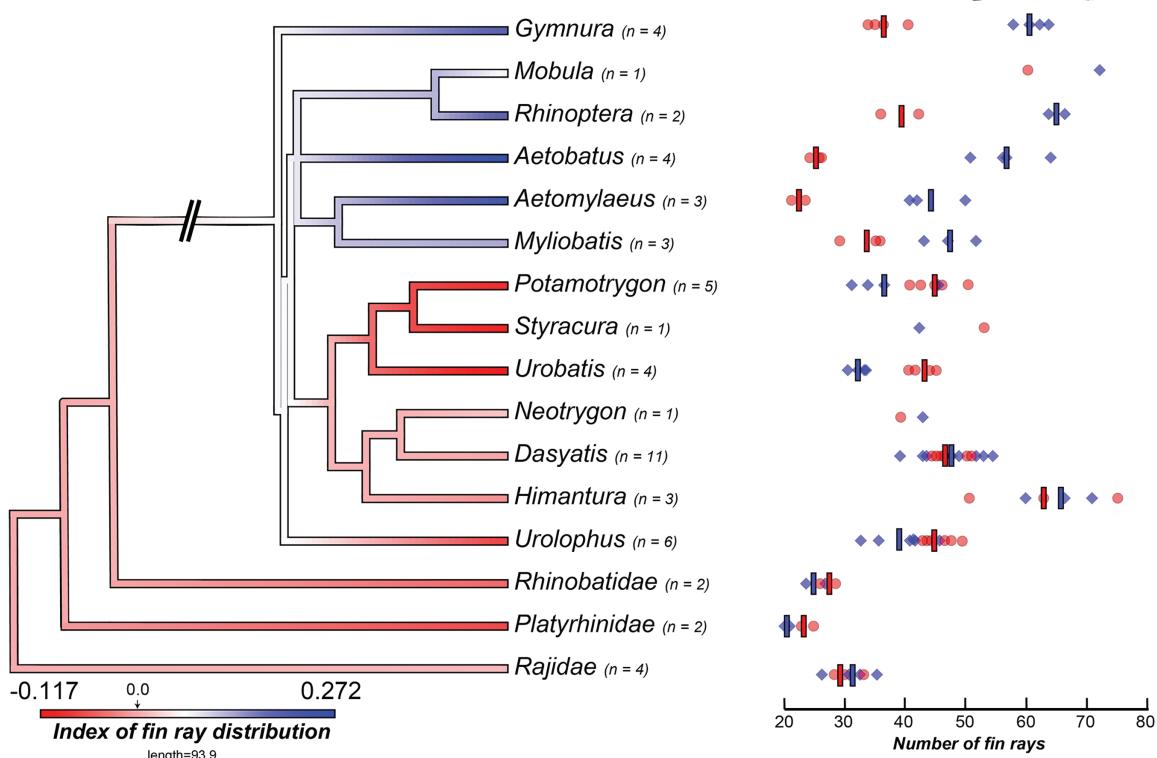
We first counted the number of fin rays articulating with primary cartilages using averages within genera (Figure 5). We performed a second analysis with multiple species to demonstrate consistency within genera (Supporting Information Figure S2). The average was taken for species with a sample size greater than one.

## 2.5 | Phylogenetic context and taxonomic sampling

We used the topology proposed by Aschliman (2011; Aschliman et al., 2012) because their taxonomic sampling was relevant to the aims of this study. Based on the mtGenome and two nuclear genes (*Rag1* and *Scfd2*), these studies indicate that the skates (Rajoidei) form a clade that is sister to the thornbacks, electric rays, guitarfishes, sawfishes, and stingrays (Aschliman et al., 2012). The stingrays (Myliobatidae and their relatives) are monophyletic, but the base of the stingray clade bursts from short branches and remains poorly resolved (see alternate topologies in Aschliman et al., 2012). It is widely accepted that the Myliobatidae is monophyletic, however, the internal relationships form a polytomy between *Myliobatis* + *Aetomylaeus* (Aschliman, 2011), *Aetobatus*, and *Rhinoptera* + *Mobula* (Aschliman et al., 2012; but see, Kirchoff, Hauffe, Stelbrink, Albrecht, & Wilke, 2017). Myliobatidae is sister to *Dasyatis* with *Neotrygon*, *Himantura* and a clade we refer to as the USP clade (*Urobatis*, *Styracura*, *Pomatotrygon*). We note that *Himantura* sensu Aschliman et al. (2012) is not monophyletic (Naylor et al., 2012), as *Himantura schmardae* (valid as *Styracura schmardae*, sensu De



**FIGURE 4** Distribution and number of pectoral fin rays throughout ontogeny in the little skate (*Leucoraja erinacea*). Number of fin rays articulating to the Propterygium, Mesopterygium and Metapterygium in the pectoral fin of developing little skates, ranging from stage 28 to pre-hatching ( $n = 11$ ) as well as mature adults ( $n = 3$ ). While there was some variation among individuals, the number of fin rays in the little skate appears to be set well before hatching, at approximately stage 32 (wingspan of 12 mm). The little skate appears to have a threshold of 60–70 total pectoral fin rays after setting early in development. Similarly, the Propterygium and Metapterygium appear to retain incredible symmetry in number of fin rays throughout ontogeny, which we correlate to an undulatory swimming mode



**FIGURE 5** Ancestral reconstruction and graph of genera fin ray index. Ancestral states reconstruction of fin ray index (Metapterygium-Propterygium/Total) for genera of batoids, represented by an average from multiple species and individuals per genus. *n* represents number of species per genus. Character state reconstructions were conducted using the Bayesian derived phylogenetic hypothesis of the Batoidea following Aschliman (2011, fig. 4.1). The ancestral state is an index of 0, being symmetric (a color close to light pink). Red colors indicate a relationship with more fin rays in the propterygium relative to the metapterygium, distinct in the USP clade and *Urolophus*. Blue colors indicate a relationship where there's more fin rays in the metapterygium than the propterygium, as seen in all oscillatory swimmers. The graph shows the average number of fin rays in the propterygium and metapterygium of families Rajidae, Platyrhinidae, and Rhinobatidae, and genera of Myliobatoidei sampled. Red circles represent the Propterygium or Propterygium + Cephalic lobe (for myliobatids). Blue diamonds represent the Metapterygium. Each circle or diamond represents a species average for that genus. The red and blue rectangle represent the average number of pectoral fin rays per primary cartilage for that genus. The Rajidae exhibit symmetry, indicated by heavy overlapping of data points. Axial swimmers (Platyrhinidae and *Rhinobatos*) and some undulatory swimmers exhibit slight anterior bias. The USP clade and *Urolophus* exhibit anterior bias, while we see a reversal in these trends by the myliobatids and gymnurids all having more in the metapterygium, which we've correlated to an oscillatory swimming lifestyle

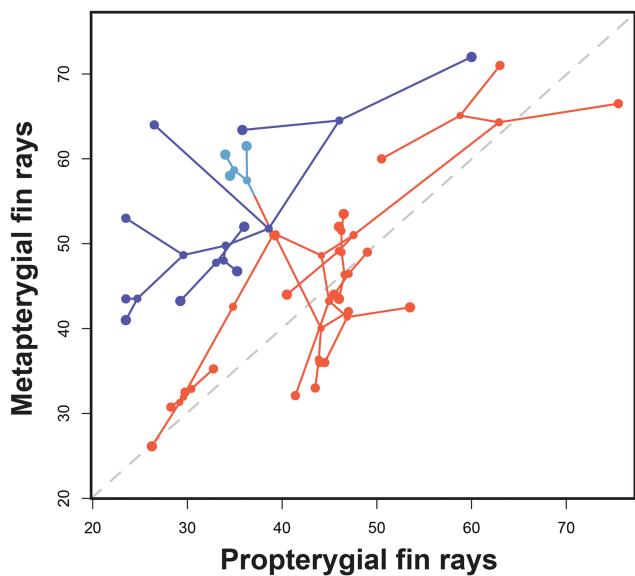
Carvalho, Loboda, & Da Silva, 2016) groups with members of the USP clade. The branching order of the remaining stingrays, *Urolophus* and *Gymnura*, is not well resolved (see Aschliman, 2011; Aschliman et al., 2012). For example Aschliman et al. (2012), joins *Gymnura* with *Urolophus*. Placement of *Gymnura* is of particular interest because they, like the Myliobatidae, exhibit oscillatory swimming and laterally expanded pectoral fins (González-Isáis & Domínguez, 2004). However, gymnurids differ from myliobatids in that they lack cephalic lobes and exhibit both oscillatory and undulatory swimming, resulting in their characterization of an “intermediate” swimming mode (Rosenberger, 2001).

To evaluate the number and distribution of fin rays in myliobatids and their relatives, we counted fin rays from 19 batoid genera (Table 1), including taxa from the Rajoidei (*Raja*, *Leucoraja*, and *Beringraja*); Platyrhinoidei (*Platyrhinoidis* and *Platyrhina*); two *Rhinobatos* species

(“Guitarfishes 2” clade, sensu Aschliman et al., 2012); and the Myliobatoidei (*Urolophus*, *Himantura*, *Dasyatis*, *Neotrygon*, *Urobatis*, *Styracura*, *Potamotrygon*, *Gymnura*, *Myliobatis*, *Aetomylaeus*, *Aetobatus*, *Rhinoptera*, and *Mobula*).

## 2.6 | Phylogenetic comparative analyses

To evaluate variation in the distribution of fin rays in a phylogenetic context, we took advantage of the topology proposed by Aschliman (2011), pruning taxa for which meristic data were lacking or unobtainable, and substituting tip taxa for closely related species when appropriate (Table 1 and Supporting Information Figure S2). All substitutions were within genera and/or placed in accordance with the most recent phylogenies (Chiquillo, Ebert, Slager, & Crow, 2014). This study focuses



**FIGURE 6** Phylomorphospace. The stochastic character histories of swimming modes, following Rosenberger (2001), simulated on a phylogeny cast into morphospace of Metapterygium and Propterygium. Myliobatidae, Gymnuridae, and the remaining rays were coded as oscillatory, oscillatory and undulatory, and undulatory, respectively. Blue correlates to the Myliobatidae, Light blue the Gymnuridae, and Red all other Myliobatoidei and Rajoidei

on species utilizing pectoral fin locomotion (i.e., the skates and rays), however we were able to obtain data for two axial-swimming taxa: thornbacks ( $n = 2$ ) and guitarfishes ( $n = 2$ ). Character state reconstructions were conducted using the Bayesian derived phylogenetic hypothesis of the Batoidea following Aschliman (2011, fig. 4.1). The topology was made ultrametric utilizing the “Chronos” command in the R program “ape” (v.4.0, Paradis, Claude, & Strimmer, 2004). We implemented fixed dates for splits derived from Aschliman et al. (2012). The new ultrametric tree was trimmed using “drop.tip” in “ape,” to remove all tips without fin ray counts. The “contMap” command in the R package “phytools” (v.0.5.20, Revell, 2012) was used to infer ancestral states of fin ray indices in a maximum likelihood framework (Figure 5).

The relationship between swimming mode and fin ray distribution was explored using multiple approaches. The stochastic character histories of swimming modes, following Rosenberger (2001), were simulated using a one-parameter model with equal rates and the “make.simmap” command in the R package “phytools”. Myliobatidae, Gymnuridae, and the remaining rays were coded as oscillatory, oscillatory and undulatory, or undulatory, respectively. Evolution of swimming mode and fin ray distributions (metapterygium and propterygium fin ray counts) were visualized using the “phylomorphospace” command in “phytools” (Revell, 2012), which projects a phylogenetic tree into morphospace (Sidlauskas, 2008). This enabled the projection of the stochastic character histories of swimming modes into the morphospace of fin ray counts (Figure 6). A maximum likelihood framework for ancestral state reconstruction of fin ray indices was accomplished using “fastAnc” command in “phytools.” The resulting estimation was then plotted as a traitgram (Evans, Smith, Flynn, & Donoghue, 2008) using the “phenogram” command in “phytools.” The traitgram also included

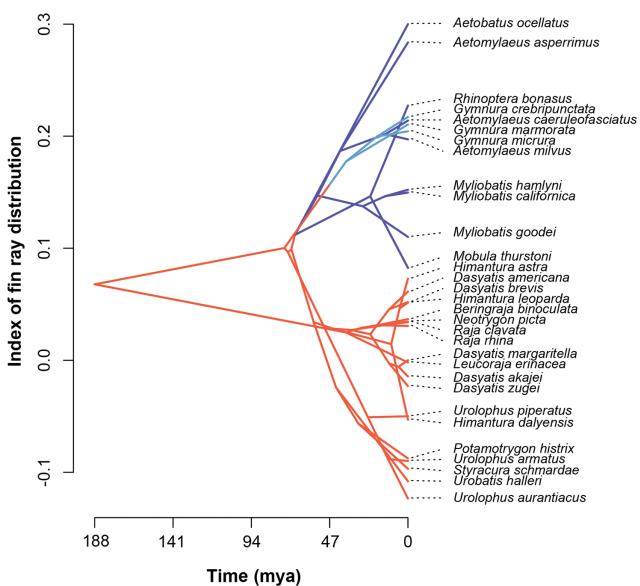
the stochastic character histories of swimming modes painted onto the phylogenetic tree. In the traitgram, phylogenetic relationships are maintained, including branches painted with swimming modes evolution, while the x-axis depicts evolutionary time and the y-axis describes variation in fin ray indices (Figure 7).

### 3 | RESULTS

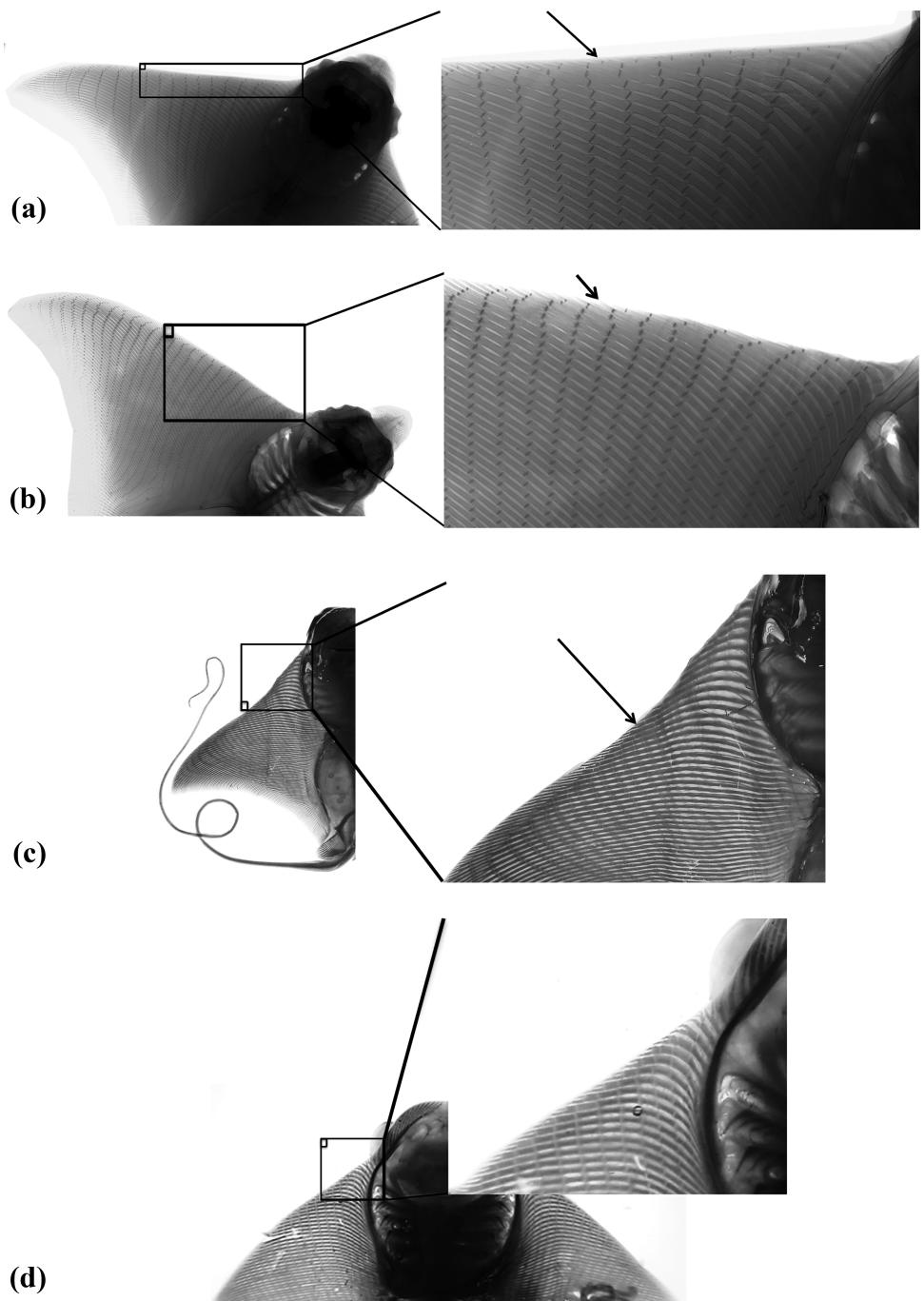
The number of fin rays articulating with the propterygium, mesopterygium and metapterygium were counted for multiple individuals representing 57 species from 19 genera (Table 1). Of the 241 individuals included with meristic data, 86 individuals from 39 species were newly characterized in this study via radiographed, CT-scanned, dissected or cleared and stained specimens. Further, most previous studies report total fin ray counts as a morphological meristic, however, by examining the distribution of fin rays with clearly defined articulation points, we were able to infer evolutionary trends and body plan remodeling associated with oscillatory swimming and the invasion of a pelagic environment.

#### 3.1 | The number of pectoral fin rays is set early in development

The total number of fin rays in the little skate, at the earliest stages of pectoral fin development (stages 28–30, following Maxwell et al., 2008), ranged from 40 to 42 rays (with a wingspan of only 5 mm), which is approximately two thirds of the number fin rays in mature adults. However, by stage 32–33, embryos with wingspans of 12–20 mm have



**FIGURE 7** Traitgram. The inferred ancestral states of fin ray index (Metapterygium-Propterygial/Total), with the stochastic character histories of swimming modes, following Rosenberger (2001), mapped on the phylogeny using the Bayesian derived phylogenetic hypothesis of the Batoidea following Aschliman (2011, fig. 4.1), and implemented fixed dates for splits derived from Aschliman et al. (2012, table 4, fig. 1). Blue correlates to the Myliobatidae, Light blue the Gymnuridae, and Red all other Myliobatoidei and Rajoidei. X-axis depicts evolutionary time, million years ago (mya)



**FIGURE 8** Variation in the *compagibus laminam*. The “jointed plate” in the anterior pectoral fin of multiple myliobatid genera (a–c) vs the uniform terminal branching of all fin rays articulating with the Cephalic lobe + Propterygium, found in two *Myliobatis* species (d). Arrow indicates where terminal branching begins for fin rays articulating with the Propterygium. A: *Aetomylaeus milvus* B: *Aetobatus narinari* C: *Rhinoptera bonasus* D: *Myliobatis californica*

similar numbers of fin rays as mature adults with wingspans of 160–234 mm or greater (with visibly mature claspers in males, Figure 4 and Supporting Information Figure S3). While there is individual variation in the total number of fin rays at stage 32 or older (ranging from 64 to 69 rays), there was no significant difference ( $p > .05$ ) between embryos (stage 32-hatching,  $n = 9$ ) and adults ( $n = 3$ ), suggesting that the number of fin rays in the little skate is set early in development, before hatching (i.e., at approximately stage 32 *sensu*, Maxwell et al., 2008).

The number of fin rays is set early in the round stingray as well. We sampled three embryos before birth with wingspans ranging from 34 to 39 mm, (newborns vary in disc width from 63 to 80 mm, Babel, 1967), one newborn (66 mm), one juvenile (115 mm), and three adults ranging from 149 to 195 mm. The number of pectoral fin rays in all individuals ( $n = 8$ ) were as follows: the propterygium 39–43, mesopterygium 12–15, metapterygium 31–35, and total number of fin rays was between 86 and 90. There was no significant difference between

TABLE 1 Number and distribution of pectoral fin rays in batoids

Genus	Species	Cephalic lobe	Propterygia	Mesopterygia	Metapterygia	Total	Index (Meta-Pro)/ Tot	C&S	X-Ray/ Dissected	CT	Species substitutions from Aschliman 2011 for Supporting Information Figure S2
<i>Leucoraja</i>	<i>erinacea</i>	0	27.5	10.7	27.5	65.7	0.000	9 <sup>1</sup>	3 <sup>2</sup>	0	<i>Leucoraja naevus</i>
<i>Raja</i>	<i>rhina</i>	0	32.8	13.8	35.3	81.9	0.031	1 <sup>1</sup>	3 <sup>2</sup>	0	X
	<i>clavata</i>	0	28.3	14.5	30.8	73.6	0.034	0	2 <sup>2</sup>	0	X
<i>Beringraja</i>	<i>binoculata</i>	0	29.8	12.8	32.5	75.1	0.036	1 <sup>1</sup>	1 <sup>2</sup>	0	<i>Dipturus batis</i>
<i>Platyrrhinoidis</i>	<i>triseriata</i>	0	25.5	6	20	51.5	-0.107	0	1 <sup>3</sup>	0	X
<i>Platyrrhina</i>	<i>exasperata</i>	0	22	12	21	55	-0.018	0	1 <sup>3</sup>	0	X
<i>Rhinobatos</i>	<i>stellio</i>	0	26	9	24	59	-0.034	0	1 <sup>4</sup>	0	X
	<i>glaucostigma</i>	0	29.5	8	27	64.5	-0.039	0	1 <sup>3</sup>	0	X
<i>Gymnura</i>	<i>marmorata</i>	0	36.3	22.3	61.5	120.1	0.210	1 <sup>1</sup>	1 <sup>4</sup>	0	<i>Gymnura zonura</i>
	<i>australis</i>	0	40.5	25	62.5	128	0.172	0	1 <sup>5</sup>	0	X
<i>crebripunctata</i>	0	34	27.5	60.5	122	0.217	0	1 <sup>2</sup>	0	X	
<i>micrura</i>	0	34.5	22.5	58	115	0.204	0	2 <sup>1,2</sup>	0	X	
<i>Urolophus</i>	<i>aurantiacus</i>	0	43.5	8.5	33	85	-0.124	0	0	1 <sup>6</sup>	<i>Urolophus cruciatus</i>
	<i>armatus</i>	0	44	9	36	89	-0.090	0	1 <sup>7</sup>	0	<i>U. paucimaculatus</i>
<i>deforgesii</i>	0	47.1	10.8	42.1	100	-0.050	0	10 <sup>7</sup>	0	X	
<i>neocaledoniensis</i>	0	48	9	41	98	-0.071	0	11 <sup>7</sup>	0	X	
<i>papilio</i>	0	49.7	10.7	46	106.4	-0.035	0	11 <sup>7</sup>	0	X	
<i>piperatus</i>	0	47	11	42	100	-0.050	0	18 <sup>7</sup>	0	<i>U. westraliensis</i>	
<i>Himantura</i>	<i>dalyensis</i>	0	75.5	27.5	67	170	-0.050	0	4 <sup>8*</sup>	0	<i>Himantura uarnacooides</i>
	<i>leoparda</i>	0	63	21.5	71	155.5	0.051	0	5 <sup>8*</sup>	0	<i>Himantura cf. gerrardi</i>
	<i>astra</i>	0	49.5	20.5	62	132	0.095	0	6 <sup>8*</sup>	0	<i>Himantura cf. uarnak</i>
<i>Dasyatis</i>	<i>brevis</i>	0	46	17.5	52	115.5	0.052	0	1 <sup>1</sup>	0	X
	<i>americana</i>	0	46.5	14.2	53.5	114.2	0.061	2 <sup>1</sup>	0	1 <sup>6</sup>	X
	<i>violacea</i>	0	45	15.5	39.5	100	-0.055	1 <sup>9</sup>	0	0	X
	<i>zugei</i>	0	46	18.5	43.5	108	-0.023	0	0	1 <sup>6</sup>	X
	<i>akajei</i>	0	45.5	14	44	103.5	-0.014	0	1 <sup>2</sup>	0	<i>Taeniura grabata</i>
	<i>margaritella</i>	0	49	20	49	118	0.000	0	1 <sup>4</sup>	0	<i>Taeniura meyenii</i>

(Continues)

TABLE 1 (Continued)

Genus	Species	Cephalic lobe	Propterygia	Mesopterygia	Metapterygia	Total	Index (Meta-Pro)/ Tot	C&S	X-Ray/ Dissected	CT	Species substitutions from Aschliman 2011 for Supporting Information Figure S2
<i>Pastinaca</i>	0	45	10.3	47.3	102.6	0.022	0	3 <sup>10</sup>	0	X	
<i>chrysonota</i>	0	46.2	14.6	48.5	109.3	0.021	0	13 <sup>10</sup>	0	X	
<i>marmorata</i>	0	46.5	14.5	48	109	0.014	0	2 <sup>10</sup>	0	X	
<i>prevircaudata</i>	0	51	14.5	52	117.5	0.009	0	17 <sup>11*</sup>	0	X	
<i>matsuburai</i>	0	55.5	16	55	126.5	-0.004	0	10 <sup>11*</sup>	0	X	
<i>Neotrygon</i>	<i>picta</i>	0	42	16	44.5	102.5	0.024	0	6 <sup>8*</sup>	0	<i>Neotrygon kuhlii</i>
<i>Urobatis</i>	<i>maculatus</i>	0	40.6	12.2	30.8	83.6	-0.117	0	3 <sup>1,2</sup>	0	X
	<i>halleri</i>	0	41.7	13	32.7	87.4	-0.103	41 <sup>1,12</sup>	4 <sup>1,2,4,12</sup>	0	X
	<i>jamaicensis</i>	0	44	12.5	33.5	90	-0.117	0	1 <sup>1</sup>	0	X
	<i>tumbesensis</i>	0	44.5	13.5	33	91	-0.126	0	1 <sup>1</sup>	0	X
<i>Styracura</i>	<i>schmidtae</i>	0	53.5	17.5	42.5	113.5	-0.097	0	1 <sup>1</sup>	0	X
<i>Potamotrygon</i>	<i>diereba</i>	0	50	21.5	45	116.5	-0.043	0	1 <sup>1</sup>	0	X
	<i>magdalenae</i>	0	40.5	13	30.5	84	-0.119	0	1 <sup>1</sup>	0	X
	<i>reticulatus</i>	0	42	15	36	93	-0.065	0	1 <sup>1</sup>	0	X
	<i>nistrix</i>	0	44.5	16.5	36	97	-0.088	0	1 <sup>2</sup>	0	X
	<i>motoro</i>	0	45.8	13.8	33.3	92.9	-0.135	0	1 <sup>1</sup>	1 <sup>6</sup>	X
<i>Myliobatis</i>	<i>californica</i>	16.3	19.8	18.9	52	107	0.149	1 <sup>12</sup>	3 <sup>12</sup>	0	<i>Myliobatis frenimillei</i>
	<i>goodei</i>	14.5	20.8	22.5	46.8	104.6	0.110	0	2 <sup>3</sup>	0	<i>Myliobatis australis</i>
	<i>hamiltoni</i>	7	20.5	19	41	87.5	0.154	0	1 <sup>5</sup>	0	X
	<i>hamiltoni</i> <i>tobijei</i>	9.5 No data	21.5 21	20 18.5	45.5 43.5	96.5 83	0.150 No data	0	12 <sup>13*</sup>	0	X
<i>Aetomylaeus</i>	<i>caeruleofasciatus</i>	7.5	14	25.5	41.2	88.2	0.223	0	2 <sup>5</sup>	0	X
	<i>caeruleofasciatus</i>	8.5	15	26.5	43.5	93.5	0.214	0	7 <sup>14*</sup>	0	X
	<i>asperimus</i>	7.5	16	27.5	53	104	0.284	0	1 <sup>4</sup>	0	X
	<i>milvus</i>	10	13.5	24.3	41	88.8	0.197	0	2 <sup>2</sup>	0	<i>Aetomylaeus nichofii</i>
<i>Aetobatus</i>	<i>narinari</i>	12	11.5	36	56	115.5	0.281	0	1 <sup>2</sup>	0	X
	<i>narutobiei</i>	12	13	33	56	114	0.272	0	3 <sup>15*</sup>	0	X

(Continues)

TABLE 1 (Continued)

Genus	species	Cephalic lobe	Propterygia	Mesopterygia	Metapterygia	Total	Index (Meta-Pro)/ Tot	C&S	X-Ray/ Dissected	CT	Species substitutions from Aschliman 2011 for Supporting Information Figure S2
	<i>flagellum</i>	14.5	12	30	51	107.5	0.228	0	8 <sup>16*</sup>	0	X
	<i>ocellatus</i>	13.5	12.5	34.5	60.5	121	0.285	0	4 <sup>17*</sup>	0	X
<i>Rhinoptera</i>	<i>bonasus</i>	19.4	16.7	22.1	62.8	121	0.221	3 <sup>12</sup>	6 <sup>18</sup>	0	<i>Rhinoptera steindachneri</i>
	<i>brasiliensis</i>	21.8	20.4	26.9	66.4	135.5	0.179	0	4 <sup>18</sup>	0	X
<i>Mobula</i>	<i>thurstoni</i>	28.5	31.5	13.5	72	145.5	0.082	1 <sup>1</sup>	0	0	<i>Manta birostris</i>

The total number of pectoral fin rays were collected and further distinguished by determining which primary cartilage each fin ray articulates with in order to understand varying distributions of pectoral fin rays for 57 species from 19 genera that exhibit different swimming modes. Columns indicate Genus and species, number of fin rays in the cephalic lobe, propterygium, mesopterygium, and metapterygium, the calculated index of fin ray distribution(meta-pro/tot, range -1 to 1), along with the method of visualization and the number of individuals for that species, superscript indicates source of specimen, asterisk indicates the median taken from previous studies and names of species substitutions found in Supporting information Figure S2. C&S short for cleared and stained, CT short for CT-scans. Bold species indicate new data characterized by this study.  
 Sources: 1. This study, Summers UW Collection C&S or X-ray 2. This study, X-ray California Academy of Sciences collection 3. This study, Online X-ray Smithsonian National Museum of Natural History 4. This study, Online X-ray California Academy of Sciences collection 5. This study, Online X-ray The Living Atlas of Australia 6. This study, Naylor CT-scan "sharkrays.org" 7. Seret et al. 2003—Description of four new stingarees of the genus *Urolophus* (Batoidea: Urolophidae) from the Coral Sea, South-West Pacific 8. Last et al. 2008—Descriptions of new Australian chondrichthyans 9. This study, Bedore GSU collection 10. Cowley et al. 1993—A taxonomic re-evaluation of the blue stingray from southern Africa (Myliobatiformes: Dasyatidae) 11. Le Port et al. 2013—Speciation of two stingrays with antitropical distributions: low levels of divergence in mitochondrial DNA and morphological characters suggest recent evolution 12. This study, Crow SFSU collection 13. White et al. 2015—Redescription of the eagle rays *Myliobatis hamlynii* Ogilby, 1911 and *M. tobijei* Bleeker, 1854 (Myliobatiformes: Myliobatidae) from the East Indo-West Pacific 14. White et al. 2016—*Aetomylaeus caeruleofasciatus*, a new species of eagle ray (Myliobatiformes: Myliobatidae) from northern Australia and New Guinea 15. White et al. 2013—A new species of eagle ray *Aetobatus narutobiei* from the northwest Pacific: An example of the critical role taxonomy plays in fisheries and ecological sciences 16. White et al. 2013—Redescription of *Aetobatus flagellum* (Bloch & Schneider, 1801), an endangered eagle ray (Myliobatidae: Myliobatidae) from the Indo-West Pacific 17. White et al. 2010—Clarification of *Aetobatus ocellatus* (Kuhl, 1823) as a valid species, and a comparison with *Aetobatus narinari* (Euphrasen, 1790) (Rajiformes: Myliobatidae) 18. Jones NOAA.

embryos ( $n = 3$ ) and adults ( $n = 3$ ), therefore, we conclude that fin ray number is set early in development, before birth in the round stingray.

Finally, the number of fin rays is similar between embryos and adults of two myliobatid species. We were able to evaluate a single embryo and three adults of the bat ray, the total number of fin rays were 106 vs. 107–108, respectively. The number of fin rays in three embryos and six adults of the cownose ray was nearly identical in the cephalic lobes, propterygium, and mesopterygium (19–20, 16–17, 21–22, respectively), with fewer articulating with the metapterygium in the embryos vs. the adults (60–61 in the embryos  $n = 3$ , vs. 62.5–65 in adults,  $n = 6$ ). Although the total number of fin rays across life stages of the cownose ray were similar, 117.5–124, we did find a significant difference between embryos and adults ( $p = .0238$ ) which we attribute to the observation that the fourth, and most distal, metapterygial TME element (and associated fin rays) had not yet developed in the embryos. For example, one embryo (wingspan of 65 mm) had three metapterygial elements and lacked the TME, while a larger and presumably older embryo (wingspan of 155 mm) had four metapterygial elements (same as adults) with fin rays articulating with the TME. These results indicate that fin ray development in the anterior 2/3rd of the pectoral fin and cephalic lobes of myliobatids (i.e., those fin rays articulating with the propterygium and mesopterygium) are set early in development, while the TME and posterior pectoral fin rays develop and become fully distinguishable later. The embryos sampled were still undergoing maturation, leaving the possibility that total number of fin rays is set before birth in these species as well.

### 3.2 | Anterior-posterior symmetry in the distribution of pectoral fin rays in skates, thornbacks, and guitarfish and inference of the ancestral batoid state

The number of fin rays articulating with the propterygium and metapterygium were nearly identical in embryos and adults ( $n = 9$  and 3, respectively) of the little skate beginning at stage 32, with an average of 27.5 fin rays articulating with the propterygium and metapterygium individually (standard deviation = 1.02 and 0.85, respectively). Fin rays are added to all three primary cartilages during early development in the little skate before the number becomes fixed (i.e., the anterior is not set before the posterior). The little skate exhibits a  $FR_{Dist}$  of 0.000, while the rajids overall ranged from 0.000 to 0.036 which is consistent with symmetry (Table 1). The four representative axial swimmers (*Platyrrhina*, *Platyrhinoidis*, and two species of *Rhinobatos* guitarfishes) exhibited symmetry or slight anterior bias ( $FR_{Dist}$  −0.107 to −0.018). The ancestral state reconstruction of fin ray indices of all 19 batoid genera indicates that the ancestral batoid exhibited symmetry (estimated  $FR_{Dist} = 0.010069$ , Figure 5, Table 1).

### 3.3 | Distribution of pectoral fin rays in stingrays and variation in AP symmetry

The stingrays appear to have diversified rapidly in association with a redistribution of fin rays (Figure 5). While three stingray genera (*Neotrygon*, *Dasyatis*, and *Himantura*) vary around symmetry, there are two major trends away from anterior-posterior symmetry: *Urolophus* and the USP clade have more fin rays distributed toward the anterior, while

*Gymnura* and the Myliobatidae have more fin rays distributed toward the posterior (Figure 5, Table 1). Specifically, the undulatory rays with anterior bias had  $FR_{Dist}$  ranging from −0.135 to −0.035, while oscillatory rays have a positive  $FR_{Dist}$  ranging from 0.082 to 0.285.

### 3.4 | Variation in cephalic lobe morphology and fin ray structure in myliobatids

The oscillatory swimmers have an increased number of fin rays articulating with the metapterygium relative to the propterygium with a  $FR_{Dist} > 0.08$ , even when fin ray counts of the cephalic lobe and propterygium are combined (Table 1). The genus *Aetobatus* has fewer, but thicker fin rays in the PPE, with 10–13% of the total number of fin rays located in the cephalic lobes. *Rhinoptera* has more cephalic lobe fin rays than *Aetobatus*, but their fin rays are not as thick, and 16% of the total number of fin rays were located in the cephalic lobes. Finally, *Mobula* have the greatest number of cephalic lobe fin rays, which are very slender and have the highest number of inter-radial fin ray joints, with 20% of the total number of fin rays located in their cephalic lobes. The mobulids have more fin rays articulating in the cephalic lobes relative to other myliobatids, while still retaining the trend of posterior bias (i.e., more fin rays articulating with the metapterygium than the propterygium and cephalic lobe combined, resulting in a lower, but still positive  $FR_{Dist}$ , Table 1, Figure 5). Therefore, our data indicate that myliobatids have more fin rays at the posterior than the anterior, despite the presence of cephalic lobes at the anterior.

### 3.5 | Variation in pectoral fin ray structure in myliobatids

There is variation in the configuration and morphology of fin rays in the anterior pectoral fin of the Myliobatidae. Schaefer and Summers (2005) observed laterally expanded radials that fit close together forming a nearly solid plate in the leading edge of oscillating wings. We further characterize this skeletal feature as the *compagibus laminam*, latin for “jointed plate.” The *compagibus laminam* is defined as a set of condensed fin rays articulating with the propterygium that have interradial fin ray joints but no terminal branching (Figure 8). This derived skeletal feature is found only in anterior pectoral fins of the Myliobatidae. However, there is variation in the presence/absence of the *compagibus laminam* within the genus *Myliobatis*, as some species exhibit distinct morphological differences in fin ray structure in the region of the *compagibus laminam*, between the cephalic lobe and anterior pectoral fin (Supporting Information Figure S4). For example, *Myliobatis hamlynii* exhibits a *compagibus laminam*, and cephalic lobe morphology similar to *Aetobatus* and *Aetomylaeus*. However, the feature is lacking in *M. californica* and *M. goodei* while *M. freminvillei* exhibits an intermediate morphology. The latter three species exhibit stunted cephalic lobe morphology that forms a continuous field in the same plane as the anterior pectoral fin, in which all fin rays exhibit terminal branching (i.e., no break in fin rays in the shoulder region, Figure 8d). The remaining Myliobatidae species of *Aetomylaeus*, *Aetobatus*, *Rhinoptera*, and *Mobula* that we evaluated do exhibit the *compagibus laminam* ( $n = 7$ ).

The posterior region of the myliobatid pectoral fin has more fin rays than the anterior, and they are more slender and elongate than the anterior and medial fin rays. These fin rays exhibit an increased number of inter-radial joints and longer radial bifurcations.

### 3.6 | Phylogenetic comparative analyses of fin ray distribution and the evolution of swimming modes

Most skates and rays utilize undulatory swimming and exhibit AP symmetry with respect to the distribution of pectoral fin rays (Figures 6 and 7). Oscillatory swimming and body plan remodeling arose twice independently; in the Myliobatidae and Gymnuridae. While pelagic stingrays (*Dasyatis violacea*) are known for their capabilities of intermediate and oscillatory swimming modes (Rosenberger, 2001) we found that their body plan, structure of pectoral fin rays and index do not deviate from the trends exhibited by all other Dasyatidae species examined. When the stochastic character of swimming modes is cast into phylomorphospace (Figure 6) and as a traitgram (Figure 7) there are distinct domains associated with fin ray distribution and swimming mode. Fin ray distribution is symmetric in undulatory rays (with  $FR_{\text{Indices}}$  varying around zero) or slightly anteriorly biased (*Urolophus* and USP clade) (Figures 5–7). Oscillatory swimmers (*Gymnura* and *Myliobatidae*) have more fin rays articulating with the metapterygium in the posterior pectoral fin.

## 4 | DISCUSSION

### 4.1 | The number of pectoral fin rays is set early in development

The number of fin rays is set early in development in all batoids examined. These findings align with the concept postulated by Romer (1950) in which “the vertebrate skeleton at an early embryonic stage is an almost perfect tiny model of that of the adult.” This suggests that the number of fin rays does not increase with allometric growth into adulthood; rather, just as chondrification of skeletal elements materialize early in development (Gillis et al., 2009) so too are the number of fin rays set before hatching or birth, after which development consists only of growth to these elements. Further, the structure and number of fin rays in the *compagibus laminam* are also set early in development in the myliobatid species that exhibit this derived framework. These findings indicate that fin ray counts from skate and stingray embryos (i.e., post-fusion of the pectoral fins) and myliobatid newborns (i.e., after birth) are representative of adults and comparable among species. This information should be useful for future studies requiring meristic data, particularly in taxa which are difficult to sample and/or are underrepresented in museum collections, which is often the case with large or rare batoid species.

### 4.2 | Cephalic lobe fin rays maintain several ancestral features

Cephalic lobes have been described as independent appendages that are optimized for feeding in myliobatids, yet we found no evidence for derived character states or evolution of additional fin rays associated

with the origin of cephalic lobes. We compared character states of pectoral fins in specific batoid taxa and sorted them by swimming mode and morphological domain (anterior pectoral fin vs. cephalic lobe). These data, representing traits that have been described in our study and others (Mulvany & Motta 2013, Sasko et al., 2006, Mulvany & Motta 2014, Schaefer & Summers 2005), highlight the retention of primarily ancestral character states in cephalic lobes with respect to distribution of Ampullae of Lorenzini, fin ray elements, and feeding functionality. The cephalic lobes of myliobatids exhibit a dense distribution of Ampullae of Lorenzini, similar to the anterior pectoral fin and mouth region of ancestral batoids (with the notable exception of *Mobula* spp., Mulvany & Motta, 2013). Further, the radials in cephalic lobe fin rays lack cross bracing, and are round in cross-section, similar to the fin rays of undulatory batoids that are flexible for feeding efficiency (Mulvany & Motta, 2013, 2014). They also exhibit crustal calcification, which is an ancestral character shared by sharks and axial swimmers (Schaefer & Summers, 2005; Underwood & Claeson, 2017). Alternatively, there are two examples of unique modifications in cephalic lobe musculature. *Rhinoptera bonasus* exhibits a specialized “levator” muscle which may be hydrodynamically beneficial as it allows the rays to retract the cephalic lobes when swimming, thereby reducing drag (Mulvany & Motta, 2013). *Mobula* species have thin dorsal oblique muscles (Mulvany & Motta, 2013), which may be beneficial for filter-feeding as they uncurl the lobes to funnel plankton into the mouth, and provide a hydrodynamic advantage when curled in their resting state (Notarbartolo-di-Sciara & Hillyer, 1989). *Aetobatus* species exhibit a stiff shovel-like cephalic lobe, and appear to have an exceptionally thick dorsal oblique muscle (Mulvany & Motta 2013). Therefore, while cephalic lobes are exclusive to the myliobatids, and exhibit derived muscular modifications, they do not appear to exhibit derived skeletal modifications. Rather, the pectoral fins of myliobatids have been divided into functionally distinct domains, and this division of labor likely released the remainder of the pectoral fins from constraints associated with feeding.

### 4.3 | Derived modifications in myliobatid pectoral fins are associated with specialization and functional adaptation

The separation of cephalic lobes from the pectoral fins in myliobatids has allowed both structures to functionally diversify. Without the constraints of prey acquisition and food processing, the pectoral fins developed a number of novel adaptations for oscillatory flight. For example, the aspect ratio of oscillatory swimmers (wingspan: body length) is 1.4 to 2.0 times greater than undulatory rays, which is associated with increased thrust and reduced drag to facilitate higher cruising speeds in pelagic environments (Fontanella et al., 2013). The anterior region of myliobatid pectoral fins is stiff relative to other batoids, with cross-bracing, increased radial thickness in the leading edge of the wing (Schaefer & Summers, 2005), and the *compagibus laminam* where propterygial pectoral fin rays lack terminal branching. This parallels the case in Labridae reef fishes, species with high aspect ratio (wing-like) pectoral fins used in the flapping (oscillatory) swimming mode are stiffer

relative to species with broader (paddle-like) fins (Aiello et al., 2018; Thorsen & Westneat, 2005).

In addition to modifications of fin ray structure in anterior pectoral fins of oscillatory swimmers, AP symmetry is broken in these groups, with more fin rays articulating in the posterior pectoral fin than the anterior. This is startling because it remains true even when cephalic lobe fin rays are counted as "anterior" and is unexpected in light of the anterior elaboration of the scapulocoracoid. We argue this is another example of morphological specialization allowing functional diversification in myliobatid wings. The thickened leading edge of the pectoral fins coupled with the wing-like taper and prominent sweepback, concentrate the surface area toward the trailing edge, shifting lift distribution posteriorly and affecting pitching equilibrium (Fish, Dong, Zhu, & Bart-Smith, 2017; Fontanella et al., 2013; Webb, 1975). Oscillatory movements are produced by anteriorly directed lift throughout the stroke to generate a thrust vector (Fish et al., 2016). We propose that the stiff anterior part of the wing is well suited for producing lift, but poorly suited to the task of guiding swimming. In the myliobatids maneuvering has been pushed rearward, where the increased number of fin rays and inter-radial joints allow for flexibility (Schaefer & Summers, 2005) and fine control, with assistance from a more angled inter-radial joint pattern converging toward the tail, thereby directing pectoral fin flexion toward the trailing edge (Russo, Blemker, Fish, & Bart-Smith, 2015). The trailing edge is where maneuverability rests in airplanes, with ailerons, rudder and elevator behind a leading airfoil, so perhaps it is no surprise that myliobatid pectoral fins have compartmentalized functionality in a similar manner. Overall, we find that while the pectoral disk of the undulatory batoids is symmetric (or nearly so) and multifunctional around the circumference, the disk of myliobatids is divided into three functional regions—the anterior cephalic lobes specialized for feeding, the stiff anterior wing for producing lift and thrust, and the flexible posterior wing for maneuverability.

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