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Autumn D. Magnuson
University of Louisville

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Diversification and convergence following the transition from saltwater to freshwater in stingrays

By
Autumn Danielle Magnuson

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University of Louisville
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Lay Summary

One of the most fundamental questions in biology is why some groups of organisms are more diverse than others. Classic hypotheses for explaining differences in diversity consider factors such as timing, new places or resources, or lack of competition. Freshwater and saltwater environments have similar levels of diversity, despite being significantly different in size, so they are useful to understand what factors influence diversity. Although transitioning from saltwater to freshwater over evolutionary time is challenging, stingrays have done this multiple times and across different continents. In this study, I evaluated how often marine stingrays have invaded freshwater, examined how separate groups of stingrays may be distinct from one another in three different ways, and assessed if freshwater stingrays have evolved to be more similar over time. I found that, like nearly all other aquatic groups, saltwater stingrays are overwhelmingly more likely to invade freshwater than freshwater stingrays are to invade saltwater. After multiple separate invasions, river rays did not have a pattern of increased diversity in the number of lineages or skeletal shapes. However, saltwater stingrays did not change in shape as predicted by a model and had spikes of increased difference in their shape around the same time as two extinction events. Although freshwater stingrays did not have a distinctive skeleton compared to saltwater stingrays, they do push the boundaries of the diversity of skeletal shapes. Stingrays sharing similar diets did not evolve similar skeletons, like how fish-eating and mollusk-eating stingrays were distinct from other groups. I did not find evidence that freshwater stingrays have evolved to be more like one another, which may be because there has not been enough time for this to occur among ancient and more recent freshwater lineages.

Abstract

One of the most fundamental questions in biology is why some groups of organisms are more diverse than others. Classic hypotheses for explaining differences in diversity consider distinctions in time, place, resources, and competitors as the staging grounds for differential diversification. Freshwater and saltwater environments have similar levels of diversity despite significant differences in size, so studying transitions between the two systems can provide insights into evolutionary processes. Despite the challenges associated with this transition, stingrays have invaded freshwater habitats multiple times across different continents, making them useful for better understanding these systems. In this study, I evaluated the frequency of saltwater-freshwater invasions in stingrays, examined three types of diversification among freshwater and saltwater stingrays, and assessed the degree of convergence among freshwater stingrays. I found that, like nearly all other aquatic taxa, stingrays overwhelmingly only transition from saltwater to freshwater. After independent freshwater invasions, river rays did not demonstrate a pattern of increasing morphological or lineage diversification. However, the phenotypic disparity of saltwater stingrays did not follow the Brownian prediction and appeared to spike around two extinction events. Despite not being morphologically distinct from saltwater stingrays, freshwater stingrays do push the boundaries of morphological diversity. Diet guilds did demonstrate morphological differences, with piscivores and molluscivores being distinct from other diet guilds. Freshwater stingrays did not appear to converge morphologically, which may be because there has not been enough time for this to occur among more ancient and more recent freshwater lineages.

Introduction

Modern biodiversity on Earth has been shaped by the evolution of taxa over millions of years. While some lineages have diversified into many species, others have not. This can be seen in the relative diversity of freshwater and saltwater fish. Saltwater covers 70% of the earth's surface, while habitable freshwater lakes and rivers make up less than 1% (Eschmeyer et al., 2010; Horn 1972; Lundberg et al., 2000; Leveque et al., 2008; Shiklomanov, 1995, pp.119-122). Despite this, species diversity in freshwater fish is comparable to that of saltwater (Leveque et al., 2008; Vermeij & Grosberg, 2010). As a result, transitions from saltwater to freshwater provide a model system for understanding why diversity is unevenly distributed between environments. When these invasions occur multiple times, they also provide an opportunity to test if evolution follows deterministic patterns where separate geographic invasions have similar outcomes (Bloom & Lovejoy, 2012, 2017).

The environmental transition from saltwater to freshwater is accompanied by both physiological stress and competition from entrenched, primary freshwater fishes (ostariophysans), which can function as ecological filters (Thorson & Watson 1975; de Brito et al., 2022). The subsequent bottleneck effect can limit the genotypic and phenotypic diversity of invaders (Kirchoff et al., 2017). However, diversity could also be shaped by convergence due to similar conditions in freshwater environments. Despite these challenges, some taxa have transitioned from saltwater to freshwater during repeat invasions, although typically not in the same geographic region (Betancur et al., 2012; Buser et al., 2019). For example, needlefishes and their allies (Beloniformes) have invaded freshwater systems six separate times and experienced similar changes in morphology following the transition, which may be related to the presence of new prey and differences in locomotor requirements (Kolmann et al., 2020). Conversely, cottoid fishes (e.g., sculpins, greenlings), which invaded freshwater at least twice, exhibit niche conservatism and fill similar ecological roles in both freshwater and saltwater environments (Buser et al., 2019). Like needlefishes and cottoids, stingray species can be found in both marine and freshwater environments due to prior invasions (Thorson & Watson, 1975; Lovejoy, 1996; Kirchhoff et al., 2017).

Over the past 90-100 million years, stingrays have evolved to fill a variety of niches in their environments (Aschliman et al., 2012). Today, there are 243 species of stingrays, which belong to 8 different families. One hundred fifty-seven of these can be categorized as dasyatoids, which include members of Dasyatidae, Urotrygonidae, and Potamotrygonidae. While many species live in marine environments or inhabit estuaries during specific stages in their life history, approximately one-fifth of species are freshwater obligates that never enter marine environments. These species can be found in Africa, Asia, and South America (Grant et al., 2019; Thorson & Watson 1975). Additionally, an extinct group of freshwater stingrays once inhabited western-central North America (de Carvalho 2004) during the early Eocene while an extant population of freshwater stingrays exists today in the St. John’s River (Florida), invading sometime during the Holocene (Bernard, 2015). Remarkably, all these regions were invaded in separate events (Kirchhoff et al., 2017). Based on osmoregulatory (Thorson & Watson, 1975), molecular (Kirchhoff et al., 2017; Kolmann et al., 2022), and morphological (Lovejoy, 1996) data, these invasions likely occurred at different points in the past, with South American stingrays likely diversifying in freshwaters earlier than other lineages and African stingrays evolving most recently. After invading freshwater, river rays in South America became ecologically and phenotypically diverse with distinct dietary niches that evolved over millions of years (Kolmann et al., 2022). Ecological, if not also morphological, convergence is suggested by the presence of insectivorous freshwater stingrays in Africa and South America (Thorson & Watson, 1975; Shibuya et al., 2009; Kolmann et al., 2016). However, whether other invasions of other freshwaters in Africa or SE Asia by stingrays have led to similar patterns of diversification is uncertain.

Here, I explored the differences in lineage, ecological, and morphological diversification among freshwater and saltwater stingrays and assessed if freshwater stingrays from different geographical regions converge with respect to diet and phenotype. To do this I: (1) compiled the existing information on stingray diet diversity using a literature review, (2) quantified how many times stingrays have invaded freshwaters using ancestral state reconstruction, and (3) evaluated differences in lineage, ecological, and morphological diversification between saltwater and freshwater stingrays, using a combination of published data and newly collected phenotypic data measured from radiographs. Finally, I (4) assessed whether, and to what extent, there is phenotypic convergence among FW stingray feeding morphologies using the distance- and morphospace-based approaches proposed by Stayton (2015). I predict that freshwater and saltwater stingrays will be morphologically distinct from one another, and separate lineages of freshwater stingrays will exhibit convergence.

Methods

I compiled existing information on stingray habitats through a literature review. After obtaining data on each species’ geographic region from FishBase (www.fishbase.se), I classified species as either freshwater (FW) or saltwater (SW) (Grant et al., 2019). Contrary to Grant et al. (2019), I classified any diadromous species as freshwater taxa, given that they spend considerable time in freshwaters and use freshwater resources. Using the phylogenetic tree proposed in Stein et al. (2015) and stochastic character mapping, a Bayesian method for ancestral state reconstruction of discrete characters, I estimated how many times stingrays have invaded freshwater environments (Bolback, 2006; Huelsenbeck et al., 2012). I contrasted the fit of three models of trait evolution: ER (equal rates of transitions), SYM (symmetrical rates), and ARD (all-rates-different), and determined which of these models best fit the data according to which had the lowest AICc score (Akaike information criterion, corrected), which represents the fit of the models. I reconstructed states at nodes using the best-fit model (ER) and 500 iterations.

A second literature review was conducted to collect data on stingray diet diversity from gut content data (Table S1). Stingray species were categorized into discrete dietary groups according to whether a given prey taxon accounted for 60% or greater of the predator’s total gut contents. If no one taxonomic group predominated (< 60%), the stingray predator was classified as an omnivore (Kolmann et al., 2022).

To compare differences in diversification between saltwater and freshwater stingrays, I combined the data obtained in the literature reviews with the radiograph measurements. I visualized lineage diversification among freshwater and saltwater taxa using lineage-through-time plots (LTT) and morphological diversification using a disparity-through-time plot (DTT). Results were analyzed using the Morphological Disparity Index (MDI), with Brownian evolution as the null model.

To examine morphological diversification, I measured 22 morphometric features from 2D and 3D radiographic datasets. I acquired both x-rays and CT scans of 106 specimens, which represented 81 dasyatoid species. 18.9% of these were freshwater and 81% were saltwater. These were imported into ImageJ and converted into 2D image files, where I measured 22 previously identified functional characters as follows (Figure 1): head length (HL), Lo, Li, gape width

(GapeW) lower minimum jaw height (LoMinJawH), lower maximum jaw height (LoMaxJawH), lower jaw length (LoJawL), upper jaw length (UpJawL), lower symphysis height (LoSymH), upper symphysis height (UpSymH), lower dentary width (LoDentW), upper dentary width (UpDentW), hyomandibula length (HyoL), minimum hyomandibula width (MinHyoH), maximum hyomandibula length (MaxHyoH), minimum propterygia width (MinPropte), maximum propterygia height (MaxPropte), propterygia arc length (PropterArc), adductor fossa area (AddFossa), and occlusional offset (OccOff). These functional characters were used to identify the anterior mechanical advantage (antMA), posterior mechanical advantage (pstMA), upper jaw aspect ratio (UJAspect), lower jaw aspect ratio (LJAspect), Hyomandibular aspect ratio (HyoAspect), and the propterygia aspect ratio (PropterAspect). Of these, 8 traits were retained for further analysis (gape width, hyomandibular offset, occlusional offset, anterior mechanical advantage, upper jaw aspect ratio, lower jaw aspect ratio, hyomandibula aspect ratio, and the propterygia aspect ratio). Each of these traits corresponds to particular mechanical features of the feeding apparatus (Dean et al., 2007; Kolmann et al., 2022).

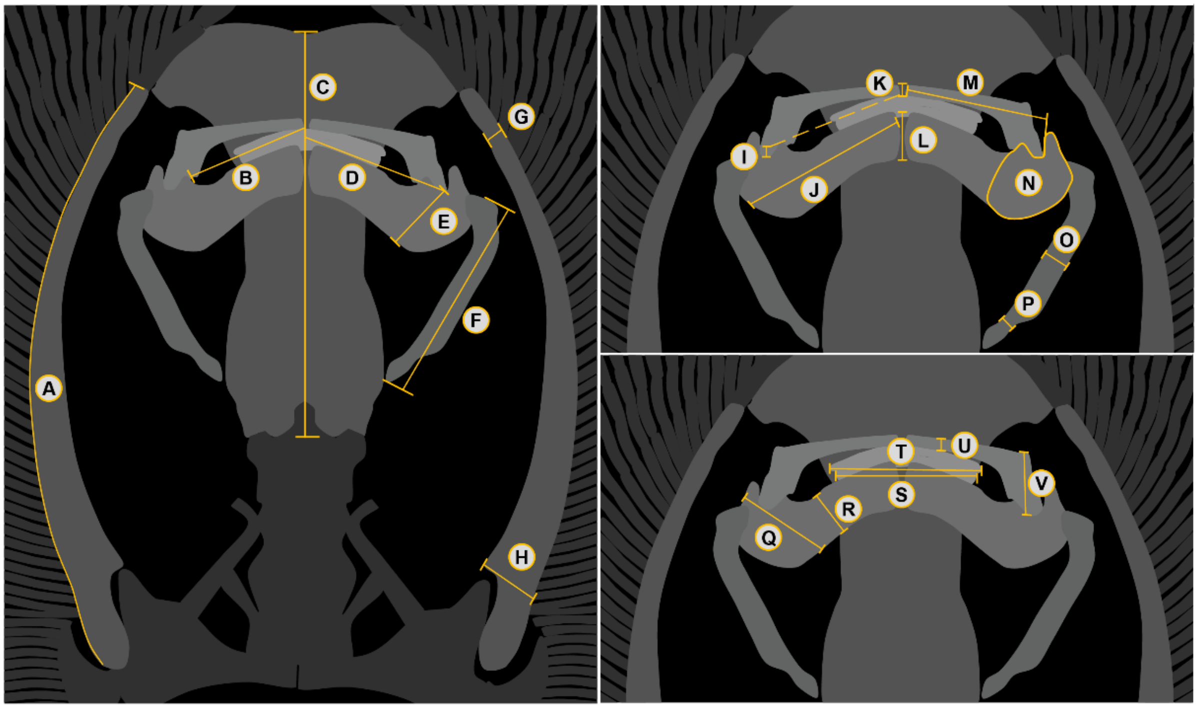

Figure 1: Diagram showing skeletal structures and measurements. The images are based on the cartilaginous skeleton of *Urobatis concentricus*. Labels represent the characters as follows: (A) Propterygia length, (B) ½ gape width, (C) head length, (D) Lo, (E) Li, (F) hyomandibula length, (G) minimum propterygia width, (H) maximum propterygia length, (I) occlusional offset, (J) lower jaw length, (K) upper symphysis height, (L) lower symphysis height, (M) upper jaw length, (N) adductor fossa area, (O) maximum hyomandibula width, (P) minimum hyomandibula length, (Q) maximum lower jaw height, (R) minimum lower jaw height, (S) lower dentary length, (T) upper dentary width, (U) minimum upper jaw height, (V) maximum upper jaw height.

The linear measurements were normalized by the head length of the stingray to correct for differences in body size. Phylogenetic methods are limited in that they can only consider a single set of values at a given tip in the phylogeny. Since I measured multiple specimens per species, I needed to reduce our dataset for inclusion with phylogenetic measures. Instead of averaging trait values across multiple specimens, I ordinated all specimens for a given species in a common morphospace using principal components analysis (PCA) in R and retained the specimen that was nearest the centroid for a given species. I chose this approach rather than averages in order to avoid unrealistic or chimeric data entries.

Next, I explored whether freshwater species occupied novel regions of phenotypic space and whether freshwater taxa are more diverse than saltwater taxa, using a phylomorphospace approach. Phylomorphospaces are projections of multidimensional trait data into a 2D space using principal components analysis (PCA) as an ordination method. For our purposes, I used a phylogenetically- informed PCA (pPCA) to form our morphospace. The phylogeny was projected onto each species' trait values to form the final phylomorphospace. If freshwater species were occupying a novel region of the phylomorphospace, their points would be clustered in a distinct region separate from the saltwater species. If they were more diverse, they would occupy a larger area. I quantified differences in diversity between freshwater and saltwater species by comparing the Procrustes variance of each group, using the *morphol.disparity* function in the geomorph package (v. 4.0.4; Adams et al., 2022). I also compared whether freshwater and saltwater stingrays overlap in their trait values using a randomized residual permutation procedure (rrpp), using habitat as our covariate. RRPP is essentially a multivariate version of analysis of variance (ANOVA) using the *lm.rrpp* function in geomorph (Dean et al., 2022). Finally, I tested whether freshwater species exhibited higher phenotypic rates of evolution than saltwater taxa using the *compare.evol.rates* function in geomorph.

To assess if there is convergence among freshwater stingrays, I used the R package convevol (Stayton, 2018) to estimate convergence using the four C metrics (C_1 – C_4) (Stayton 2015). C_1 is defined as one minus the distance between the tips of the compared phylogenetic groups in a morphospace divided by the greatest distance between their ancestral nodes, where a value of one would indicate complete convergence and zero would indicate a complete absence of convergence. C_2 is used to account for the magnitude of change and calculate C_3 and C_4. C_3 reveals the degree of convergence across the entire evolutionary history of the phylogeny used and C_4 describes the convergence within the evaluated clade. For both C_3 and C_4, a value closer to one suggests that a greater degree of a group's evolutionary history has been shaped by convergence. If there is convergence among freshwater stingrays, they would be morphologically similar and occupy similar regions in the morphospace, resulting in high C-values.

Results

Literature Review

Data on the diet of stingrays were obtained for 68 species of stingrays and 1 species of panray through 60 previously published papers found in the literature review. Data on habitat was obtained for 226 species through FishBase (www.fishbase.se). The 52 species that were classified as freshwater were further investigated through 11 additional published sources (Table S1).

Ancestral State Reconstruction

The ER (AICc = 49.48, log-likelihood = -23.71) model was favored over the symmetric model (SYM) and all rates different model (ARD). Transition frequencies between freshwater and saltwater were biased, where transitions from saltwater to freshwater (likelihood = 6.518) were over 7.5 times more likely than from freshwater to saltwater (likelihood = 0.848).

These ancestral state reconstructions found that the transition from saltwater to freshwater has occurred at least six times, with South America being invaded once, Africa being invaded twice, and Asia being invaded three times (Figure 2). The earliest invasion was in South America, with subsequent invasions in Africa and Asia occurring more recently. The multiple invasions of freshwaters in Africa and Asia do not appear to have happened concurrently. Older invasions of Asia occurred in the *H. signifier* + *H. oxyrhyncha* clade, with a more recent invasion by *Dasyatis laosensis*. Likewise, African freshwaters were invaded first by *Urogymnus ukpam* and then more recently by *Dasyatis garouaensis*.

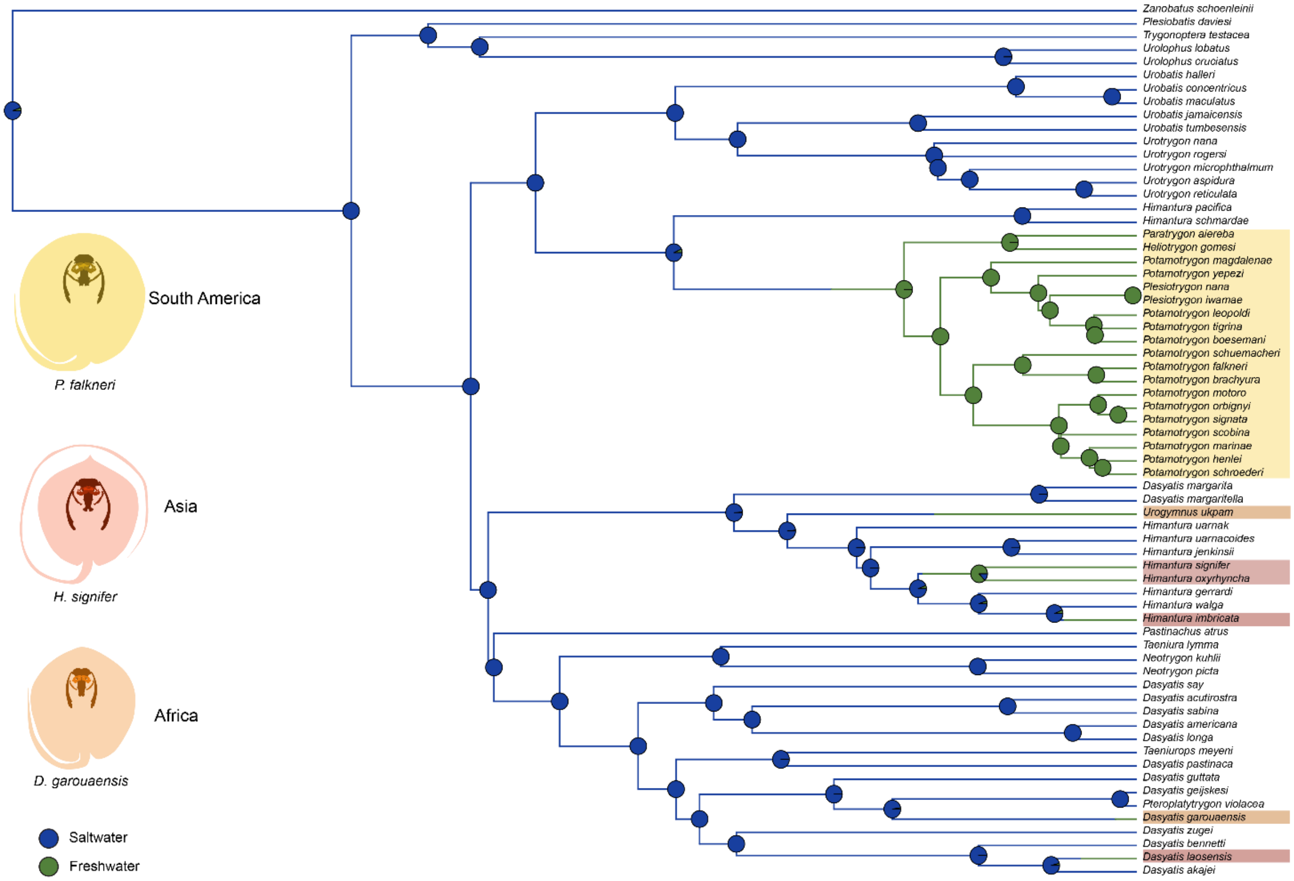

Figure 2. Stingrays have transitioned from saltwater to freshwater 6 times. Phylogeny with stochastic character mapping for the ancestral state reconstruction of saltwater and freshwater stingrays. Pie charts on nodes represent the frequency that the node was designated as freshwater or saltwater. Dark blue designates saltwater groups and green represents freshwater groups. Stingray diagrams show freshwater species from different geographic regions and are colored to match the highlighted taxa from the same region.

Lineage Diversification

Based on the results of the lineage-through-time plots, freshwater stingrays experienced a linear increase in lineage diversification beginning between approximately 60 and 75 million years ago (mya). Starting around 40 mya, lineage diversification began to increase, and by about

10 mya it became exponential ($\gamma = 1.33$, $p = 0.18$). Saltwater stingrays began to diversify approximately 120 mya. Diversification shifted towards exponential three times, at approximately 100 mya, 65 mya, and 35 mya ($\gamma = -1.77$, $p = 0.076$). After each period of a substantial increase in diversification, lineage diversification would abruptly slow until the next exponential curve began (Figure 4).

In saltwater stingrays, phenotypic disparity followed a similar pattern to lineage diversification. About 99 mya, phenotypic disparity became slightly greater than expected from the Brownian model. This timing coincides with the Bonarelli Event. At around 66 mya, the same time as the end of the Cretaceous-Paleogene extinction event, there was a second spike where disparity became greater than expected. Freshwater stingrays had a slight deviation from the confidence intervals at about 20 mya, but this was not significantly different from the Brownian expectation (MDI = 0.1751, $p = 0.175$).

Morphological Diversification

The first two principal component axes (PCs) of the PCA plot represent >50% of the variance in the data (PC1: 33.9% and PC2: 18.3%) (Table 1). PC1 is primarily characterized by the robustness, mechanical advantage (leverage), and occlusion of the jaws; jaw protrusion based on the proxy hyomandibular offset; and gape width. PC2 represents the strength of the propterygia through the proxy measurement of the propterygia aspect along with the robustness of the hyomandibula and jaws through the hyomandibular aspect and jaw aspect ratios, respectively. PC3 (13.1% of the variance) most strongly summarizes the gape width, hyomandibula aspect ratio (HyoAspect), and propterygia aspect ratio (PropterAspect).

Table 1. Principal component (PC) axes loadings for PCs 1-3. **Bolded** text load positively on respective axis.

	PC1 (33.9%)	**PC2 (18.3%)**	**PC3 (13.1%)**
Gape Width (mouth width)	-0.6842021	-0.01575705	**0.5680008**
Hyomandibular Offset	-0.5212562	**0.05727597**	-0.1989238
Occlusional Offset	-0.7877352	**0.26137444**	-0.0150674
Mechanical Advantage (jaw leverage)	-0.6377095	-0.21937373	-0.3119837
Upper Jaw Aspect Ratio	-0.7537206	**0.31865337**	**0.229867**
Lower Jaw Aspect Ratio	-0.4611431	-0.68360518	**0.101065**
Hyomandibular Aspect Ratio	**0.2785971**	-0.62696416	**0.5464825**
Propterygial Aspect Ratio	**0.2982164**	**0.61927434**	**0.4752439**

Freshwater and saltwater stingrays occupy much of the same space in the pPCA (Figure 3). As a result, there is no significant difference in feeding morphologies between the two groups ($p = 0.37$, $r^2 = 0.011$). However, several stingrays have more extreme or specialized morphological characters that expand the range of their habitat type's region past the overlapping zone. Among saltwater stingrays, these include the sharpsnout stingray *Dasyatis geijskesi* and pelagic stingray *Pteroplatytrygon violacea*. The freshwater stingrays demonstrate substantial diversity in morphology and narrowly expand the envelope of the morphospace past the saltwater stingrays in all four quadrants. This is largely due to the presence of ecomorphological specialist species, such as *Paratrygon aiereba* and *Heliotrygon gomesi* (both piscivores), and *Potamotrygon leopoldi* (a molluscivore).

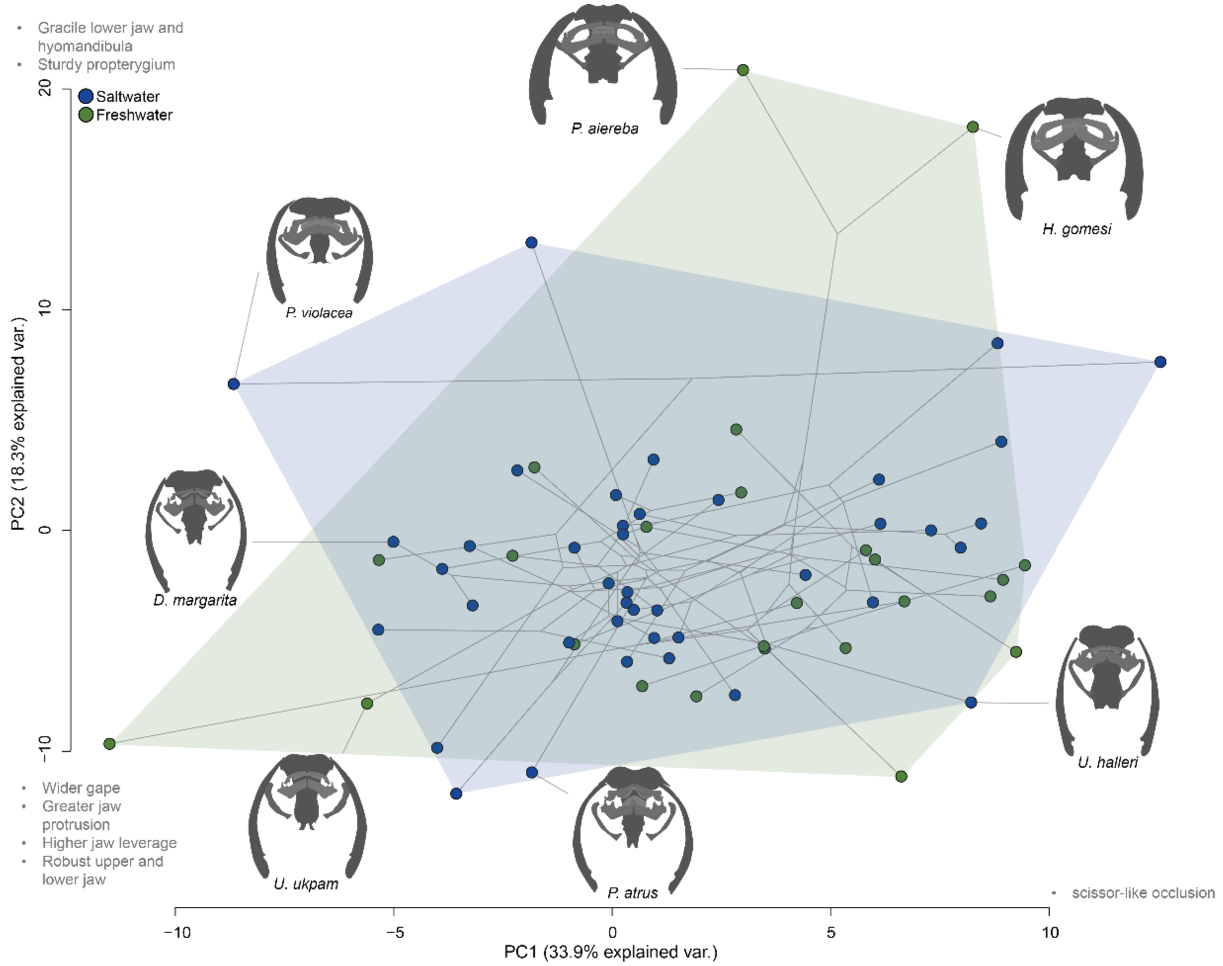

Figure 3. Freshwater stingrays expand the envelope of the phylomorphospace. Points, which are colored according to saltwater or freshwater habitat type, represent the principal component score (PC) of a species and lines represent the phylogeny of the taxa. Convex hulls outline taxa based on habitat type. The skulls of 7 representative stingrays, each from a different family, illustrate the differences in morphology across the space. The representative stingrays are *Dasyatis margarita*, *Pteroplatytrygon violacea*, *Paratrygon aierba*, *Heliotrygon gomesi*, *Urobatis halleri*, *Pastinachus atrus*, and *Urogymnus ukpam*.

While freshwater and saltwater stingrays do occupy similar regions of the morphospace, they differ in their relative total morphological disparity and respective rates of phenotypic evolution. The Procrustes variance for freshwater stingrays, which is a multivariate estimation of disparity, was two times higher than that of saltwater stingrays (173.6 vs. 87.69, respectively). The pairwise difference between the variances was 85.99 with a p-value of 0.002, indicating that the difference was significant.

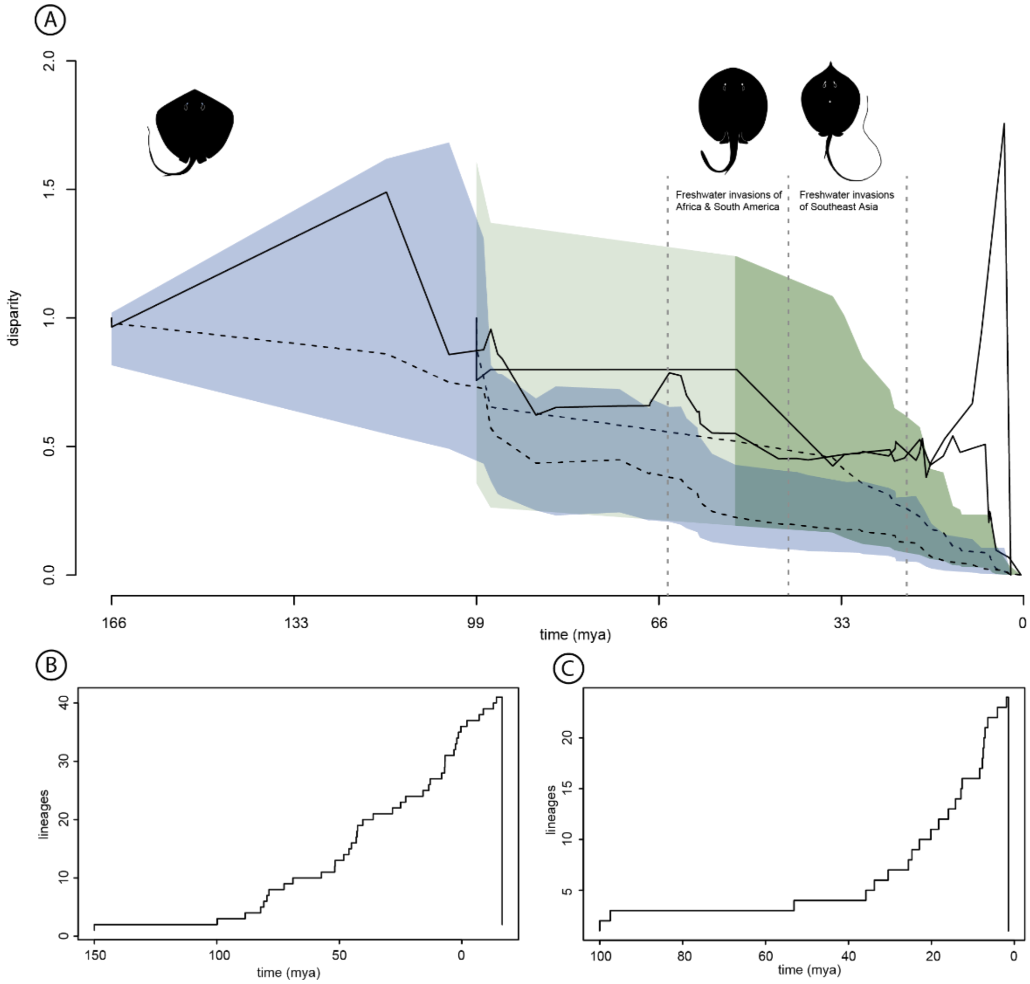

Figure 4. Freshwater and saltwater lineage diversification patterns. (A) Combined diversity through time (DTT) plot for freshwater and saltwater stingrays. The confidence interval is green for freshwater stingrays and blue for saltwater stingrays. Vertical dashed lines represent invasion events and black outlines of stingrays display examples of how taxa from the different areas appear. (B) Lineage through time (LTT) plot for freshwater stingrays. (C) LTT for saltwater stingrays. Time in all plots is in millions of years ago (mya).

Ecological Diversification

The PCA displayed substantial overlap between most diet guilds across the center of the plot (Figure 5). However, they do differ from one another morphologically (p = 0.005) The guild with the greatest area was made up of species with an unknown diet, while the guild with the least area was invertivores. Piscivores occupy a distinct region of morphospace associated with the positive loadings on PC2. This region is associated with a sturdy propterygium. Molluscivores are also distinct from the other groups, with *Potamotrygon leopoldi* extending the region in the lower left quadrant. This area is associated with thick, robust jaws and high mechanical advantage (high leverage) jaws.

The differentiation of piscivores and molluscivores from the other diet guilds was confirmed by the rrpp test results, with molluscivores found to be significantly distinct from all other groups (Table 2). Similarly, piscivores differed from all other diet guilds, with the sole exception of invertivores (p = 0.075) (Table 2). Additionally, freshwater stingrays evolved at nearly three times the rate of saltwater stingrays (rate = 2.9099, p = 0.001).

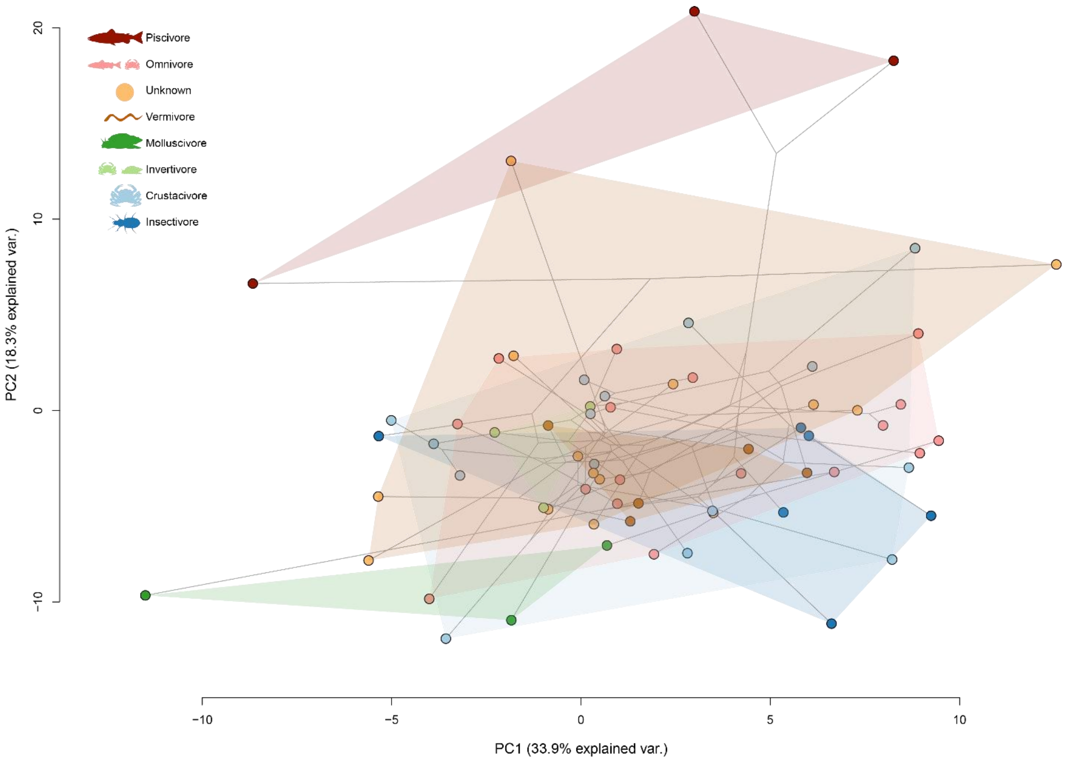

Figure 5. Phylomorphospace with groupings according to dietary guilds. Guilds are represented by different colors, with a convex hull wrapping dietary groups. The legend displays the color associated with each guild on a pictogram representing the type of organisms consumed.

Convergent Evolution in Freshwater Stingrays

The data did not suggest that there is much convergence among freshwater taxa, using any of the four C metrics. I found that evolution reduced the distance between freshwater lineages by 20.7% (C_1 = 0.2075) within the phylomorphospace, which represents 9.07% (C_2 = 2.069, C_3 = 0.09069) of the total evolution among the lineages and >1% (C_4 = 0.005226) of within the clade.

Table 2. Results of pairwise distances (d) with the 95% upper confidence interval (UCL), standard score (Z), and the p-value associated with d (p-value) for diet guilds. Rows with statistically significant differences are highlighted with p-values in bold.

	d	**UCL (95%)**	**Z**	**p-value**
Crustacivore:Invertivore	0.008232	0.054184	-0.9208998	0.83
Crustacivore:Molluscivore	0.068059	0.05366616	2.0900915	**0.005**
Crustacivore:Omnivore	0.010289	0.02228	-0.1006252	0.515
Crustacivore:Piscivore	0.047475	0.04171508	1.8982652	**0.035**
Crustacivore:Unknown	0.00644	0.023173	-0.8490897	0.8
Crustacivore:Vermivore	0.006799	0.037951	-1.1200271	0.86
Insectivore:Invertivore	0.011058	0.049595	-0.8199882	0.795
Insectivore:Molluscivore	0.069424	0.04949899	2.2064317	**0.01**
Insectivore:Omnivore	0.007188	0.033993	-1.0116453	0.82
Insectivore:Piscivore	0.05453	0.04317233	2.0173555	**0.02**
Insectivore:Unknown	0.006565	0.032602	-1.3278179	0.905
Insectivore:Vermivore	0.00782	0.037708	-1.3822085	0.9
Invertivore:Molluscivore	0.061306	0.05772572	1.7482551	**0.03**
Invertivore:Omnivore	0.012064	0.052052	-0.4578541	0.635
Invertivore:Piscivore	0.044635	0.05236381	1.4074216	0.075
Invertivore:Unknown	0.011758	0.054536	-0.4323084	0.655
Invertivore:Vermivore	0.011017	0.052333	-0.8204743	0.78
Molluscivore:Omnivore	0.068123	0.04971371	2.2286825	**0.005**
Molluscivore:Piscivore	0.055658	0.05566770	1.7672474	0.055
Molluscivore:Unknown	0.072211	0.05143609	2.1499279	**0.005**
Molluscivore:Vermivore	0.069539	0.05142084	2.0760121	**0.015**
Omnivore:Piscivore	0.055517	0.04259811	2.1233547	**0.03**
Omnivore:Unknown	0.009304	0.022415	-0.3827701	0.635
Omnivore:Vermivore	0.006357	0.037587	-1.5575493	0.92
Piscivore:Unknown	0.052669	0.04080694	2.1317477	**0.03**
Piscivore:Vermivore	0.052353	0.04839446	1.7703032	**0.04**
Unknown:Vermivore	0.00644	0.036491	-1.2809458	0.915

Discussion

I found that stingrays have invaded freshwater at least six times, which aligned with prior estimates overall (Kirchhoff et al., 2017; Kolmann et al., 2020). However, after invasions of freshwater, river stingrays exhibited little if any increase in patterns of lineage and morphological diversity relative to saltwater stingrays (Figure 3). Contrary to our hypothesis that freshwater stingrays would be morphologically distinct from marine stingrays, there was substantial overlap among most of the morphospace (but see below). Although there was no overall morphological distinction between freshwater and saltwater rays, there were overt morphological differences among diet guilds, where piscivores and molluscivorous were distinct from other guilds. However, freshwater stingrays did not exhibit convergence, which may be related to time.

Freshwater stingrays exhibit greater diversity than saltwater stingrays

Despite their later appearance in the phylogeny and significant overlap with saltwater species, freshwater stingrays are more morphologically diverse than saltwater stingrays in all quadrants of the PCA plot. This indicates that the transition from saltwater to freshwater may have resulted in increased morphological rates of diversification after invasions (Kolmann et al., 2022). However, the greater diversity in freshwater species is not associated with overall differences in morphology between the two groups – a shift in which region of morphospace one group occupies adjacent to the other. Instead, freshwater stingrays have simply expanded the established boundaries of the morphospace. These pioneering freshwater species typically were specialist taxa occupying novel ecological niches (piscivores, molluscivores) and were almost always potamotrygonids from South America, the oldest freshwater lineage. The sole exception to this is *Urogymnus ukpam*, a comparably aged freshwater stingray from western Africa.

Interestingly, the one region of morphospace not explored by saltwater stingrays may not have always been so. Members of obligate freshwater genera *Paratrygon* and *Heliotrygon* always occupy morphospace regions adjacent to saltwater stingrays in the PCA plot, except for the piscivores *Paratrygon aiereba* and *Heliotrygon gomesi*. This same area may have contained *Lessiniabatis aenigmatica*, an extinct saltwater stingray that superficially converges with *Paratrygon* and *Heliotrygon* (e.g., with large pectoral propterygia, wide mouths). However, this body plan has been lost to saltwater taxa since *Lessiniabatis* is not known to have lived after the Eocene (Marramà et al., 2019).

Molluscivores and piscivores were morphologically distinct from other diet guilds. This same trend was observed in Kolmann et al. (2022) for potamotrygonids, although the observed differences may be driven by the inclusion of potamotrygonids in this study, which are more diverse than other freshwater rays. Piscivores were primarily located in a region associated with strong pectoral propterygia. These cartilaginous elements anchor the muscles used to lift the pectoral fins. Stingrays capture prey by generating suction with their pectoral fins, lifting the fins to suck prey beneath the body (Wilga et al., 2012). By having stronger propterygia, stingray species can generate more suction and capture more elusive prey. This is especially important for piscivorous stingrays. Molluscivorous stingrays are associated with robust upper and lower jaws along with a high mechanical advantage. Because they eat prey with hard shells, such as sea snails or bivalves, having the ability to generate enough force to crush food items without damaging their jaw is crucial. The distinction of molluscivores was largely driven by *Potamotrygon leopoldi*, which specializes in hard-shelled prey (Rutledge et al., 2019).

Based on these results, it may be possible to identify some species with an unknown diet as molluscivores and piscivores. For example, the morphology of mesopelagic stingray *Plesiobatis daviesi* suggests that it is a piscivore.

Timing of diversification

There was an observed bias in the directionality of freshwater-saltwater invasions, where transitions from saltwater to freshwater were substantially more likely to occur than from freshwater to saltwater. This trend seems to be a conserved feature of animals transitioning between these two environments, which are always biased from saltwater to freshwater (except in ariid catfishes, see Betancur et al., 2012; Bloom et al., 2013). This is particularly true for other South American marine-derived freshwater fishes like needlefishes (Kolmann et al., 2020), anchovies, herring, shad, drum, and pufferfishes, which never re-invaded saltwater (Bloom & Lovejoy, 2012, 2017; Santini et al., 2013; Boeger et al., 2015). Like many of these other freshwater lineages, in river stingrays, there seems to be little clear evidence for explosive diversification after invading a novel habitat (Bloom & Lovejoy, 2012; Kolmann et al., 2022). This does not appear to align, at least closely, with ecological opportunity theory, where invading taxa radiate as they diversify into new niches (Simpson 1953; Schluter 2000; de Brito et al., 2022). Although this trend has been observed in some taxa, such as grunters (Davis et al., 2012), the opposite has occurred in other groups, potentially because of competition (Bloom and Lovejoy, 2017; de Brito et al., 2022; Santini et al., 2013).

Competition appears to be lacking for stingrays, as there are few if any other animals capable of competing with their benthic niche (Kolmann et al., 2022); instead, time for diversification might be limiting the potential for ecological opportunity in the younger African and Asian radiations. Although this study suggests that freshwater rays show some evidence of increased lineage diversification relative to saltwater relatives, this has not led to considerable lineage diversification (Bloom et al., 2013) in Asia or Africa, and the only evidence for extensive phenotypic diversification has occurred in South American potamotrygonids (Kirchoff et al., 2017; Kolmann et al., 2022).

While the disparity of freshwater stingrays has changed over time in a manner that aligns with Brownian expectations, saltwater stingrays have not. Spikes in disparity occurred twice, each around the same time as an extinction event. Previous work has found that speciation increased in batoids around the Cretaceous-Tertiary (KT) extinction event boundary, possibly due to adaptive radiation following ecological or competitive release (Aschliman et al., 2012). This trend has also been observed in other taxa and during other events (Brusatte et al., 2015; López-Estrada et al., 2019; Sidor et al., 2013; Yoder et al., 2010). However, dynamics following extinction events are complex, and release alone may not be adequate to explain the observed changes (Bapst et al., 2012; Crowley et al., 2012; Crisp and Cook, 2009). Our finding that saltwater stingrays experienced an increase in lineage diversification around the KT boundary aligns with the findings of Aschliman et al. (2012).

The smaller spike in lineage diversity occurred around the same time as the Bonarelli event, also known as Ocean Anoxic Event 2 (OAE2) (Selby and Condon 2009). This global event was associated with significant disruptions to both the carbon cycle (Karkitsios et al., 2007) and the phosphorous cycle (Papadomanolaki et al., 2022), which coupled with the rising sea levels during the mid-Cretaceous (Haq, 2014), resulted in the extinction of several marine reptile species such as ichthyosaurs (Fischer, 2016) and a strong decline in tethysuchians, a clade of crocodylomorphs (Jouve and Jalil, 2020). While the effect of OAE2 on chondrichthyans varied based on geographic location (Guinot, 2013), after the event some batoids such as sharks, experienced increased levels of phenotypic disparity (Bazzi and Siversson, 2022). It is possible that saltwater stingrays also experienced competitive release associated with OAE2, but additional research would be necessary to evaluate the timing and potential contributing factors.

Conclusions

Although it is possible that the challenges associated with living in a freshwater environment are not strong selective pressures for a particular morphotype in stingrays, the observed disparity among freshwater stingrays may be related to the timing of each diversification event. If divergence times are the reason that I did not observe convergence, I would expect that the stingrays that most recent freshwater invaders would have the least diversity and would be the most like their marine sister taxa because there has not been as much time to diversify (Bloom et al., 2013). Conversely, stingrays that invaded freshwater earlier would have a greater period to diversify and become more distinct from their closest saltwater relatives (Buser et al., 2019). I found that older freshwater invaders appear to be more diverse than freshwater stingray lineages from southeast Asia, which invaded more recently, and are the most like their saltwater sister taxa. This supports the possibility that convergence has not had enough time to occur.

Table S1. Habitat and diet of dasyatoid stingrays and sister panray. Habitat sources in *italics* were obtained from FishBase references and sources in regular I obtained from the greater literature review.

Family	Species	Habitat	Diet	Habitat Sources	Diet Sources
Dasyatidae	*Bathytoshia brevicaudata*	SW		*Compagno et al., 1989*	
Dasyatidae	*Bathytoshia centroura*	SW	Omnivore	*Bernardes et al, 2005*	Hess, 1961
Dasyatidae	*Bathytoshia lata*	SW	Crustacivore	*Last et al., 2016*	Dale et al., 2011
Dasyatidae	*Brevitrygon heterura*	SW	Crustacivore	*Last et al., 2016*	Lim et al., 2019
Dasyatidae	*Brevitrygon imbricata*	SW	Omnivore	Rainboth, 1996	Devadoss, 1983
Dasyatidae	*Brevitrygon javaensis*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Brevitrygon walga*	SW	Crustacivore	*IUCN, 2020*	Raje, 2007
Dasyatidae	*Dasyatis brevis*	SW		*De la Cruz Aguero et al., 1997*	
Dasyatidae	*Dasyatis chrysonota*	SW	Omnivore	*Last et al., 2016*	Ebert & Cowley, 2003
Dasyatidae	*Dasyatis hastata*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Dasyatis hypostigma*	SW	Microcrustacivore	*Santos and Carvalho, 2004*	Ruocco and Lucifora, 2016
Dasyatidae	*Dasyatis marmorata*	SW		*Capapé and Desoutter, 1990*	
Dasyatidae	*Dasyatis multispinosa*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Dasyatis pastinaca*	SW	Crustacivore	*Brito, 1991*	Ismen, 2003
Dasyatidae	*Dasyatis tortonesei*	SW		*Hureau and Monod, 1979*	
Dasyatidae	*Fluivtrygon oxyrhyncha*	FW		Iqbal et al., 2018	
Dasyatidae	*Fluvitrygon kittipongi*	FW		Iqbal et al., 2018	
Dasyatidae	*Fluvitrygon signifer*	FW		Compagno & Roberts, 1982	
Dasyatidae	*Fontitrygon colarensis*	SW		*Santos et al., 2004*	
Dasyatidae	*Fontitrygon garouaensis*	FW	Insectivore	Jabado, 2021	Thorson & Watson, 1975
Dasyatidae	*Fontitrygon geijskesi*	SW		*Last et al., 2016*	
Dasyatidae	*Fontitrygon margarita*	SW	Crustacivore	*Capapé and Desoutter, 1990*	Omotosho & Oyebanji, 1996
Dasyatidae	*Fontitrygon margaritella*	SW	Omnivore	*Last et al., 2016*	Clements et al., 2022
Dasyatidae	*Fontitrygon ukpam*	FW		Grant et al., 2019	
Dasyatidae	*Hemitrygon akajei*	SW	Omnivore	*Riede, 2004*	Taniuchi & Shimizu, 1993
Dasyatidae	*Hemitrygon bennettii*	SW	Omnivore	*Fricke et al., 2011*	Lim et al., 2019
Dasyatidae	*Hemitrygon fluviorum*	SW	Crustacivore	*Fricke et al., 2011*	Last & Stevens, 2009; Pierce et al., 2011
Dasyatidae	*Hemitrygon izuensis*	SW		*Last et al., 2016*	
Dasyatidae	*Hemitrygon laevigata*	SW		*Last et al., 2016*	
Dasyatidae	*Hemitrygon laosensis*	FW	Invertivore	Grant et al., 2019; Rainboth, 1996	Rainboth, 1996
Dasyatidae	*Hemitrygon longicauda*	SW		*Last and White, 2013*	
Dasyatidae	*Hemitrygon navarrae*	SW		*Last et al., 2016*	
Dasyatidae	*Hemitrygon parvonigra*	SW		*Last et al., 2016*	
Dasyatidae	*Hemitrygon sinensis*	SW		*Last et al., 2016*	
Dasyatidae	*Himantura alcockii*	SW	Omnivore	*Froese, R. and D. Pauly, 2022*	Devadoss, 1982
Dasyatidae	*Himantura australis*	SW		*Last et al., 2016*	
Dasyatidae	*Himantura fava*	SW		*Froese, R. and D. Pauly, 2022*	

Dasyatidae	*Himantura fluviatilis*	FW		*Riede, 2004*	
Dasyatidae	*Himantura krempfi*	FW	Crustacivore	Compagno & Roberts, 1982; Rainboth, 1996	Funicelli, 1975; Hess, 1961; Snelson, 1981
Dasyatidae	*Himantura leoparda*	SW		*Manjaji-Matsumoto and Last, 2008*	
Dasyatidae	*Himantura marginata*	SW		*Riede, 2004*	
Dasyatidae	*Himantura microphthalma*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Himantura pareh*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Himantura tutul*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Himantura uarnak*	SW	Omnivore	*Riede, 2004*	Devadoss, 1981; O'Shea et al., 2013; Raje, 2007
Dasyatidae	*Himantura undulata*	SW		*Froese, R. and D. Pauly, 2022*	
Dasyatidae	*Hypanus americanus*	SW	Omnivore	*Uyeno et al., 1983*	Gilliam & Sullivan, 1993
Dasyatidae	*Hypanus berthalutzae*	SW	Piscivore	*Froese, R. and D. Pauly, 2022*	Queiroz et al., 2022
Dasyatidae	*Hypanus dipterurus*	SW	Crustacivore	*Mundy, 2005*	Navarro-Gonzalez et al., 2012
Dasyatidae	*Hypanus guttatus*	SW	Omnivore	*Uyeno et al., 1983*	de Carvalho 2001; Queiroz et al., 2022
Dasyatidae	*Hypanus longus*	SW	Omnivore	*Last et al., 2016*	Lopez-Garcia et al., 2012; Navia et al., 2007
Dasyatidae	*Hypanus marianae*	SW	Crustacivore	*Gomes et al., 2000*	Queiroz et al., 2022; Shibuya & Rosa., 2011
Dasyatidae	*Hypanus rudis*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Hypanus sabinus*	FW		Grant et al., 2019	
Dasyatidae	*Hypanus say*	SW	Crustacivore	*FMNH, 2015*	Funicelli, 1975; Hess, 1961
Dasyatidae	*Maculabatis ambigua*	SW		*Last et al., 2016*	
Dasyatidae	*Maculabatis arabica*	SW		*Manjaji-Matsumoto and Last, 2016*	
Dasyatidae	*Maculabatis astra*	SW	Crustacivore	*Last et al., 2008*	Jacobsen & Bennett, 2011
Dasyatidae	*Maculabatis bineeshi*	SW		*Manjaji-Matsumoto and Last, 2016*	
Dasyatidae	*Maculabatis gerrardi*	SW	Crustacivore	*Compagno et al., 1989*	Rastgoo et al., 2018
Dasyatidae	*Maculabatis pastinacoides*	SW		*White et al., 2006*	
Dasyatidae	*Maculabatis randalli*	SW		Last et al., 2012	Rastgoo et al., 2018
Dasyatidae	*Maculabatis toshi*	SW	Crustacivore	*Last and Stevens, 1994*	Brewer et al., 1991
Dasyatidae	*Makararaja chindwinensis*	FW		Grant et al., 2019	
Dasyatidae	*Megatrygon microps*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Neotrygon annotata*	SW	Omnivore	*Last and Stevens, 1994*	Brewer et al., 1991; Jacobsen & Bennett, 2011
Dasyatidae	*Neotrygon australiae*	SW		*Last et al., 2016*	
Dasyatidae	*Neotrygon caeruleopunctata*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Neotrygon indica*	SW		*Pavan-Kumar et al., 2018*	
Dasyatidae	*Neotrygon kuhlii*	SW	Vermivore	*Weigmann, 2011*	O'Shea et al., 2013
Dasyatidae	*Neotrygon leylandi*	SW		*Last and Compagno. 1999*	
Dasyatidae	*Neotrygon ningalooensis*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Neotrygon orientalis*	SW		*Froese and Pauly, 2022*	

Dasyatidae	*Neotrygon picta*	SW		*Froese and Pauly, 2022*	Jacobsen & Bennett, 2011
Dasyatidae	*Neotrygon varidens*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Pastinachus ater*	SW	Molluscivore	*Froese and Pauly, 2022*	Jacobsen & Bennett, 2011; O'Shea et al., 2013
Dasyatidae	*Pastinachus gracilicaudus*	SW		*Allen and Erdmann, 2012*	
Dasyatidae	*Pastinachus sephen*	FW	Molluscivore	Monkolprasit, & Roberts, 1990	Devadoss, 1983; Raje, 2007; Salini et al., 1990
Dasyatidae	*Pastinachus solocirostris*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Pastinachus stellurostris*	FW		Grant et al., 2019	
Dasyatidae	*Pateobatis bleekeri*	SW		*Riede, 2004*	
Dasyatidae	*Pateobatis fai*	SW		*Fricke et al., 2011*	
Dasyatidae	*Pateobatis hortlei*	SW		*Last et al., 2006*	
Dasyatidae	*Pateobatis jenkinsii*	SW		*Last and Compagno. 1999*	
Dasyatidae	*Pateobatis uarnacoides*	SW	Crustacivore	*White et al., 2006*	Raje, 2007
Dasyatidae	*Pteroplatytrygon violacea*	SW	Piscivore	*Mundy, 2005*	Lipej et al., 2013
Dasyatidae	*Taeniura grabata*	SW		*Brito, 1991*	
Dasyatidae	*Taeniura lessoni*	SW		*Last et al., 2016*	
Dasyatidae	*Taeniura lymma*	SW	Vermivore	*Last and Compagno. 1999*	O'Shea et al., 2013
Dasyatidae	*Taeniurops meyeni*	SW		*Myers, 1999*	
Dasyatidae	*Telatrygon acutirostra*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Telatrygon biasa*	SW	Crustacivore	*Last et al., 2016*	Lim et al., 2019
Dasyatidae	*Telatrygon crozieri*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Telatrygon zugei*	SW		Riede, 2004	
Dasyatidae	*Urogymnus acanthobothrium*	SW		*Last et al., 2016*	
Dasyatidae	*Urogymnus asperrimus*	SW	Vermivore	*Fricke et al., 2011*	O'Shea et al., 2013
Dasyatidae	*Urogymnus dalyensis*	FW	Omnivore	Grant et al., 2019	Last & Stevens, 2009
Dasyatidae	*Urogymnus granulatus*	SW	Omnivore	*Last and Compagno. 1999*	Ishihara et al., 1993
Dasyatidae	*Urogymnus lobistoma*	SW		*Froese and Pauly, 2022*	
Dasyatidae	*Urogymnus polylepis*	FW	Omnivore	Grant et al., 2019; Monkolprasit, & Roberts, 1990	Sen et al., 2022
Hexatrygonidae	*Hexatrygon bickelli*	SW		*Froese and Pauly, 2022*	
Plesiobatidae	*Plesiobatis daviesi*	SW		*Mundy, 2005*	
Potamotrygonidae	*Heliotrygon gomesi*	FW		de Carvalho & Lovejoy, 2011; Grant et al., 2019	
Potamotrygonidae	*Heliotrygon rosai*	FW		de Carvalho & Lovejoy, 2011; Grant et al., 2019	
Potamotrygonidae	*Paratrygon aiereba*	FW	Piscivore	Grant et al., 2019	Shibuya et al., 2012
Potamotrygonidae	*Paratrygon orinocensis*	FW		Loboda, et al., 2021	
Potamotrygonidae	*Paratrygon parvaspina*	FW		Loboda, et al., 2021	
Potamotrygonidae	*Plesiotrygon iwamae*	FW	Crustacivore	Grant et al., 2019; Rosa et al., 1987	Charvet-Almeida, 2001
Potamotrygonidae	*Plesiotrygon nana*	FW		de Carvalho & Ragno, 2011; Grant et al., 2019	
Potamotrygonidae	*Potamotrygon adamastor*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon albimaculata*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon amandae*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon amazona*	FW		Grant et al., 2019	

Potamotrygonidae	*Potamotrygon boesemani*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon brachyura*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon constellata*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon falkneri*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon garmani*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon henlei*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon histrix*	FW	Omnivore	Driedzic and Fonesca de Almeida-Val, 1996	Shibuya & Rosa., 2011
Potamotrygonidae	*Potamotrygon humerosa*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon hystrix*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon jabuti*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon leopoldi*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon limai*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon magdalenae*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon marinae*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon motoro*	FW	Crustacivore	Grant et al., 2019	Shibuya et al., 2009
Potamotrygonidae	*Potamotrygon ocellata*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon orbignyi*	FW	Insectivore	Grant et al., 2019	Moro et al., 2011; Shibuya et al., 2010
Potamotrygonidae	*Potamotrygon pantanensis*	FW	Insectivore	Grant et al., 2019	Lonardoni et al., 2006; Silva & Uieda, 2007
Potamotrygonidae	*Potamotrygon rex*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon schroederi*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon schuhmacheri*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon scobina*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon signata*	FW	Insectivore	Grant et al., 2019	Moro et al., 2012
Potamotrygonidae	*Potamotrygon tatianae*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon tigrina*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon wallacei*	FW		Grant et al., 2019	
Potamotrygonidae	*Potamotrygon yepezi*	FW		Grant et al., 2019	
Potamotrygonidae	*Styracura schmardae*	SW	Omnivore	*Froese and Pauly, 2022*	O'Shea et al., 2020
Potamotrygonidae	*Styracura pacifica*	SW		*Froese and Pauly, 2022*	
Urolophidae	*Spinilophus armatus*	SW		*Froese and Pauly, 2022*	
Urolophidae	*Trygonoptera galba*	SW		*Last and Yearsley, 2008*	
Urolophidae	*Trygonoptera imitata*	SW		*Yearsley etal., 2008*	
Urolophidae	*Trygonoptera mucosa*	SW		*Last and Stevens, 1994*	Platell et al 1998
Urolophidae	*Trygonoptera ovalis*	SW		*Michael, 1993*	
Urolophidae	*Trygonoptera personata*	SW		*Last and Stevens, 1994*	Platell et al 1998
Urolophidae	*Trygonoptera testacea*	SW	Vermivore	*Compagno, 1997b*	Marshall et al., 2008
Urolophidae	*Urolophus aurantiacus*	SW		*Froese and Pauly, 2022*	
Urolophidae	*Urolophus bucculentus*	SW		*Compagno, 1997b*	
Urolophidae	*Urolophus circularis*	SW		*Last and Stevens, 1994*	
Urolophidae	*Urolophus cruciatus*	SW	Crustacivore	*Last and Stevens, 1994*	Treloar & Laurenson, 2006; Yick et al., 2011
Urolophidae	*Urolophus deforgesi*	SW		*Froese and Pauly, 2022*	
Urolophidae	*Urolophus expansus*	SW		*Last and Stevens, 1994*	Treloar & Laurenson, 2006
Urolophidae	*Urolophus flavomosaicus*	SW		*Compagno, 1997b*	
Urolophidae	*Urolophus gigas*	SW		*Last and Stevens, 1994*	

Urolophidae	*Urolophus javanicus*	SW		*Froese and Pauly, 2022*	
Urolophidae	*Urolophus kaianus*	SW		*Compagno, 1997b*	
Urolophidae	*Urolophus kapalensis*	SW	Omnivore	*Yearsley and Last, 2006*	Marshall et al., 2008
Urolophidae	*Urolophus lobatus*	SW		*Last and Stevens, 1994*	Platell et al 1998
Urolophidae	*Urolophus mitosis*	SW		*Last and Stevens, 1994*	
Urolophidae	*Urolophus neocaledoniensis*	SW		*Séret and Last, 2003*	
Urolophidae	*Urolophus orarius*	SW		*Last and Stevens, 1994*	
Urolophidae	*Urolophus papilio*	SW		*Séret and Last, 2003*	
Urolophidae	*Urolophus paucimaculatus*	SW	Omnivore	*Michael, 1993*	Edwards, 1980; Platell et al 1998
Urolophidae	*Urolophus piperatus*	SW		*Séret and Last, 2003*	
Urolophidae	*Urolophus sufflavus*	SW		*Campagno, 1997b*	
Urolophidae	*Urolophus viridis*	SW		*Campagno, 1997b*	
Urolophidae	*Urolophus westraliensis*	SW		*Last and Stevens, 1994*	
Urotrygonidae	*Urobatis concentricus*	SW		*Love et al., 2005*	
Urotrygonidae	*Urobatis halleri*	SW	Crustacivore	*Michael, 1993*	Flores-Ortega et al., 2011
Urotrygonidae	*Urobatis jamaicensis*	SW	Vermivore	*Lieske and Myers, 1994*	O'Shea et al., 2017; Quin 1996
Urotrygonidae	*Urobatis maculatus*	SW	Invertivore	*Love et al., 2005*	Arreguín-Sánchez et al., 2007
Urotrygonidae	*Urobatis marmoratus*	SW		*Froese and Pauly, 2022*	
Urotrygonidae	*Urobatis pardalis*	SW		*del Moral-Flores et al., 2015*	
Urotrygonidae	*Urobatis tumbesensis*	SW		*Froese and Pauly, 2022*	
Urotrygonidae	*Urotrygon aspidura*	SW	Crustacivore	*Love et al., 2005*	Navarro-Gonzalez et al., 2012
Urotrygonidae	*Urotrygon caudispinosus*	SW		*Froese and Pauly, 2022*	
Urotrygonidae	*Urotrygon chilensis*	SW	Omnivore	*Froese and Pauly, 2022*	Muro-Torres et al., 2019
Urotrygonidae	*Urotrygon cimar*	SW		*López and Bussing, 1998*	
Urotrygonidae	*Urotrygon microphthalmum*	SW	Crustacivore	*Froese and Pauly, 2022*	Santander-Neto et al., 2021
Urotrygonidae	*Urotrygon munda*	SW	Crustacivore	*Love et al., 2005*	Flores-Ortega et al., 2011
Urotrygonidae	*Urotrygon nana*	SW	Omnivore	*Froese and Pauly, 2022*	Navarro-Gonzalez et al 2012
Urotrygonidae	*Urotrygon peruanus*	SW		*Froese and Pauly, 2022*	
Urotrygonidae	*Urotrygon reticulata*	SW	Invertivore	*Froese and Pauly, 2022*	Muro-Torres et al., 2019
Urotrygonidae	*Urotrygon rogersi*	SW	Crustacivore	*Love et al., 2005*	Pierce et al., 2011
Urotrygonidae	*Urotrygon serrula*	SW		*Froese and Pauly, 2022*	
Urotrygonidae	*Urotrygon simulatrix*	SW		*Froese and Pauly, 2022*	
Urotrygonidae	*Urotrygon venezuelae*	SW		*Froese and Pauly, 2022*	
Zanobatidae	*Zanobatus schoenleinii*	SW	Omnivore	*Reiner, 1996*	Patokina and Litvinov, 2005

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