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Do key innovations unlock diversification? A case-study on the morphological and ecological impact of pharyngognathy in acanthomorph fishes

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Abstract

Key innovations may allow lineages access to new resources and facilitate the invasion of new adaptive zones, potentially influencing diversification patterns. Many studies have focused on the impact of key innovations on speciation rates, but far less is known about how they influence phenotypic rates and patterns of ecomorphological diversification. We use the repeated evolution of pharyngognathy within acanthomorph fishes, a commonly cited key innovation, as a case study to explore the predictions of key innovation theory. Specifically, we investigate whether transitions to pharyngognathy led to shifts in the rate of phenotypic evolution, as well as shifts and/or expansion in the occupation of morphological and dietary space, using a dataset of 8 morphological traits measured across 3,853 species of Acanthomorpha. Analyzing the 6 evolutionarily independent pharyngognathous clades together, we found no evidence to support pharyngognathy as a key innovation; however, comparisons between individual pharyngognathous lineages and their sister clades did reveal some consistent patterns. In morphospace, most pharyngognathous clades cluster in areas that correspond to deeper-bodied morphologies relative to their sister clades, while occupying greater areas in dietary space that reflects a more diversified diet. Additionally, both Cichlidae and Labridae exhibited higher univariate rates of phenotypic evolution compared with their closest relatives. However, few of these results were exceptional relative to our null models. Our results suggest that transitions to pharyngognathy may only be advantageous when combined with additional ecological or intrinsic factors, illustrating the importance of accounting for lineage-specific effects when testing key innovation hypotheses. Moreover, the challenges we experienced formulating informative comparisons, despite the ideal evolutionary scenario of multiple independent evolutionary origins of pharyngognathous clades, illustrates the complexities involved in quantifying the impact of key innovations. Given the issues of lineage specific effects and rate heterogeneity at macroevolutionary scales we observed, we suggest a reassessment of the expected impacts of key innovations may be warranted.

Key words: Acanthomorpha, comparative methods, ecomorphological diversification, evolutionary innovations, phenotypic rates

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Evolutionary innovations are frequently cited as drivers of phenotypic and lineage diversification across the tree of life. Innovations have been proposed to play a role in adaptive radiations by facilitating access to new resources and thus the invasion of new adaptive zones (Simpson 1944; Simpson 1953). By releasing lineages from previous evolutionary constraints, evolutionary novelties may allow shifts and/or expansion into new areas of phenotypic and ecological space (Heard and Hauser 1995; Bond and Opell 1998; Rabosky 2017). The emergence of evolutionary innovations can also promote differential evolutionary success among lineages, which is usually measured by the impact of a given trait on speciation rates, net diversification (e.g., Mitter et al. 1988; Heard and Hauser 1995; Sanderson and Donoghue 1996; Vermeij 2001) or ecomorphological evolution (e.g., Garcia-Porta and Ord 2013; Maia et al. 2013). In such cases, evolutionary innovations are usually referred to as "key innovations" (Marazzi and Sanderson 2010; Alfaro 2014).

The operationalization of the term "key innovation" (*sensu* Rabosky 2017) has contributed to an increasing number of empirical studies testing the effects of traits on taxonomic richness or lineage diversification rates (e.g., Mitter et al. 1988; Alfaro et al. 2009; Lynch 2009; Dumont et al. 2012; Rainford et al. 2014; Fernández-Mazuecos et al. 2019). However, far less is known about how key innovations influence phenotypic diversification. Indeed, little progress has been made since Schluter (2000) stated "the lack of attention to effects of novel traits on ecological and phenotypic expansion is an outstanding gap in the study of key innovations." To fully understand the role of a trait as a key innovation, researchers need to look beyond lineage diversification rates and explore whether the emergence of a trait may have affected phenotypic rates, as well as ecological and phenotypic space occupation (Hunter 1998; Rabosky 2017; Wainwright and Longo 2017). Given that the emergence of a key innovation can increase the availability of ecological resources, allowing lineages to rapidly expand into new ecological niches, we expect that the evolution of a key innovation should lead to a shift, and most likely an increase, in rates of phenotypic diversification. Some studies have found evidence for key innovations leading to increased rates of morphological evolution (Garcia-Porta and Ord 2013; Maia et al. 2013). In contrast, others have suggested they may instead constrain morphological diversification (Holliday and Steppan 2004; Ord et al. 2020). Moreover, the impact of the evolution of novel phenotypes on ecomorphological diversification may depend on a variety of biotic and abiotic factors (Wainwright and Price 2016).

In addition to rate shifts, another possible outcome of the emergence of a key innovation may be differences in patterns of morphological and ecological space occupation. By facilitating access to previously unavailable adaptive zones (Simpson 1944; Simpson 1953; Heard and Hauser 1995; Hunter 1998; Vermeij 2001), key innovations can lead to shifts and/or expansion of morpho- and ecospace occupation (Bond and Opell 1998; Rabosky 2017). Shifts refer to the exploration of distinct regions of that space by lineages possessing the key innovation relative to their ancestors, a pattern that may be difficult to observe when data for extinct lineages are lacking (Sanderson and Donoghue 1996). However, sister clades have a shared evolutionary history and presumably similar evolutionary potential upon divergence. Therefore, if the key innovation in question unites a clade of taxa, valuable insights can be gained regarding its effects on morphological and ecological diversification by making comparisons with the sister clade (Mitter et al. 1988; Slowinski and Guyer 1993; Sanderson and Donoghue 1996; Bond and Opell 1998; Vermeij 2001; Holliday and Steppan 2004). Moreover, given the

same amount of time, lineages with a key innovation are predicted to occupy a greater area of morpho- or ecospace relative to clades lacking the trait, leading to an expansion in morphological and ecological space. Although sister clades have a shared divergence time, they may substantially differ in their respective crown ages and hence the amount of time for trait diversification. Therefore, it may be more appropriate to quantify expansions in morphological and ecological space by comparing clades with similar crown ages.

While it may appear relatively straightforward to test these predictions about the macroevolutionary consequences of a hypothesized key innovation, there are several complications to bear in mind. To confer some statistical rigor and avoid spurious correlations when applying phylogenetic comparative methods to test key innovation hypotheses, it is necessary for the focal trait to have evolved several times independently within the group of interest (Slowinski and Guyer 1993; Heard and Hauser 1995; Goudet 1999; Vermeij 2001; Holliday and Steppan 2004). It is expected that all lineages possessing the key innovation will share a common signal (e.g., increased phenotypic rates) that is related to the emergence of the trait. However, diversification dynamics may be shaped by additional factors, which might differ across clades (Bond and Opell 1998; Hunter 1998). It would therefore be unsurprising if a key innovation does not have the same effect on every lineage (Hunter 1998). Understanding which ecological scenarios, or in which circumstances, a given key innovation confers an evolutionary advantage would be a fruitful step towards a more comprehensive key innovation theory.

The relationship between diversity and key innovations can be complex, and the diversification models presently available only partially account for this complexity (Alfaro 2014). Key innovation hypotheses are often tested by comparing evolutionary rates or diversity between clades with and without the trait of interest (Mitter et al. 1988; Vermeij 2001; Marazzi and Sanderson 2010; Maia et al. 2013). One common approach for these tests is to compare models allowing for rate shifts whenever the key innovation evolves on the phylogeny to a null model where a single evolutionary rate is applied across the entire phylogenetic tree (Sanderson and Donoghue 1996). However, a major caveat of this approach is that rates of ecomorphological and lineage evolution can be highly heterogeneous across evolutionary radiations (e.g., Rabosky et al. 2013; Wang and Lloyd 2016; Cooney et al. 2017) and, thus, fitting a single-rate null model to the entire phylogeny is almost certainly unrealistic (Rabosky and Goldberg 2015; Beaulieu and O'Meara 2016; Chira and Thomas 2016). These simplistic models cause problems for identifying the impact of key innovations as more complex models, which allow rates to vary between lineages with and without the innovation, may be favored simply because they account for some rate variation (Rabosky and Goldberg 2015; Beaulieu and O'Meara 2016; Caetano et al. 2018; May and Moore 2020).

Our goal for this study is to investigate pharyngognathy as a key innovation, testing the predicted outcomes across multiple macroevolutionary axes (i.e., phenotypic rates, disparity, ecological, and morphological space). Pharyngognathy was first described in the Cichlidae, arguably one of the most diverse families of fishes, by Liem (1973) who hypothesized that their novel pharyngeal complex allowed them to radiate into numerous new adaptive zones and could therefore be considered a key innovation. Since then, pharyngognathy has become a classic example of a key innovation and is frequently cited as such in scientific articles and books about evolutionary innovations and adaptive radiations (e.g., Heard and Hauser 1995; Hunter 1998; Schluter 2000). Pharyngognathy

comprises a set of novelties to the secondary jaw structures of fishes (Liem 1973; Wainwright and Longo 2017). Specifically, it is characterized by: (1) fusion or intimate suturing of the left and right fifth ceratobranchials (i.e., the lower pharyngeal jaw bones) into a robust plate-like structure; (2) a muscular sling that suspends the fused plate from the neurocranium; and (3) well-developed joints between the dorsal surface of the upper pharyngeal jaw bones and a raised protuberance on the underside of the neurocranium (Kaufman and Liem 1982; Wainwright et al. 2012; Wainwright and Longo 2017). These structural modifications are hypothesized to increase jaw strength during prey processing, broadening the range of accessible prey items (Liem 1973). For instance, it has been shown that pharyngognathous taxa transition to durophagous diets at a higher rate than non-pharyngognathous taxa (McGee et al. 2015). The increased functional capacity of the pharyngeal jaws to process prey in pharyngognathous taxa is thought to facilitate the decoupling of prey capture and prey processing, thereby freeing the oral jaws to become more specialized in capture (Liem 1973; Hulsey 2006; Burress 2016; Wainwright and Longo 2017).

The evolution of pharyngognathy among acanthomorph fishes provides an ideal opportunity to test hypotheses about key innovations. Pharyngognathy has evolved 6 times in Acanthomorpha at the base of: (1) Labridae (wrasses plus parrotfishes and weed whitings); (2) Pomacentridae (damselfishes); (3) Embiotocidae (surfperches); (4) Exocoetidae + Hemiramphidae (flying fishes and halfbeaks); (5) Centrogenyidae (false scorpionfishes); and (6) Cichlidae (cichlids) (Wainwright et al. 2012). The effects of the emergence of pharyngognathy on the macroevolutionary dynamics of fishes has been investigated in terms of lineage diversification rates, with studies finding mixed support for an overall increase in these rates (Alfaro et al. 2009; McGee et al. 2015). However, no studies to date have focused on the impact of pharyngognathy on morphological and ecological diversification across Acanthomorpha (but see Burress 2016 for a study with cichlids).

Inspired by key innovation theory, we explore three major predictions concerning the impact of pharyngognathy on phenotypic and ecological diversification within acanthomorph fishes. The first prediction we address is that transitions to pharyngognathy have led to a consistent shift in the rate of phenotypic evolution. We also investigate whether the evolution of pharyngognathy has led to shifts and/or expansion in morpho- and ecospace (diet) occupation compared with non-pharyngognathous clades. Specifically, we test the prediction that pharyngognathous lineages occupy distinct regions of morphological and dietary space relative to their sister clades. Finally, because pharyngognathy is hypothesized to increase dietary versatility, we investigate the prediction that pharyngognathous lineages have expanded their occupation of morphological and dietary space relative to non-pharyngognathous clades. We also highlight the difficulties that we encountered in testing these predictions and provide suggestions that we hope may serve as useful guidelines for future investigations on the macroevolutionary consequences of key innovations.

Materials and Methods

Morphological and diet data

We collected morphological data from preserved specimens deposited in the ichthyological collections of the Smithsonian National Museum of Natural History. The dataset comprises 3,853 species of acanthomorph fishes that are part of a larger morphometric dataset assembled across 6,144 species of teleosts (described in Price et al.

2019). We restricted our analyses to the Acanthomorpha because all transitions to pharyngognathy (as defined above) occur within this clade [Centrogenyidae ($n = 1$), Cichlidae ($n = 203$), Embiotocidae ($n = 15$), Exocoetidae ($n = 28$), Hemiramphidae ($n = 12$), Labridae ($n = 209$, includes Odacinae and Scarinae), and Pomacentridae ($n = 151$)]. Using up to three specimens per species, we calculated species averages for 8 morphological traits that are considered informative on foraging and trophic ecology: standard length, maximum body depth, maximum fish width, head depth, lower jaw length, mouth width, minimum caudal peduncle depth, and minimum caudal peduncle width. Species were selected to match the tips of a recent large-scale molecular phylogeny of ray-finned fishes (Rabosky et al. 2018); a pruned version of this tree was used for all comparative analyses.

Prior to analysis, we size-standardized the species' average linear measurements using the log-shape ratio method (Mosimann 1970; Mosimann and James 1979). This method was selected because it removes only the isometric component of shape variation, therefore preserving differences in shape due to evolutionary allometry in the data (Claude 2013; Klingenberg 2016). Briefly, we divided each morphological trait by the geometric mean of the three linear measurements most indicative of the overall size of our specimens (standard length, maximum body depth, and maximum fish width) and log-transformed the resulting ratios, yielding the size-standardized log-shape ratios.

We extracted diet data as the presence (1) or absence (0) of dietary items from Fishbase (Froese and Pauly 2020) using the rfishbase package (Boettiger et al. 2012). Diet variables were consolidated taxonomically by subphylum, superclass, or class to improve the standardization of differences between major diet items and their relative weights in the ordination analyses (see below; Supplementary Table S1). Species that returned 0's for all diet variables were considered to have no data and were removed from the ecological dataset. Our full ecological dataset comprised diet information for 2001 acanthomorph species.

Comparative methods

Testing for differences in phenotypic rates

We used stochastic character mapping to reconstruct the evolutionary history of pharyngognathy across acanthomorphs. Stochastic character mapping was implemented in the R package phytools (Revell 2012) using the *make.simmap* function with the all rates different model. The prior for the root state (pi) was determined by estimating its stationary distribution conditional on the Q-matrix. We initially produced 100 stochastic character maps that consistently showed 6 independent transitions to pharyngognathy. Given that all stochastic character maps differed only in the placement of the transitions along the stem of pharyngognathous clades, a single map was selected for further analysis (Figure 1A).

To determine if there were differences in univariate rates of morphological evolution between pharyngognathous and non-pharyngognathous clades, we used a model-fitting approach implemented in the R package OUwie (Beaulieu et al. 2012). For each morphological trait, we compared the fit of two Brownian Motion models: a single-rate model and a model estimating separate rates for pharyngognathous and non-pharyngognathous taxa. Transitions to pharyngognathy were identified according to the stochastic character map. Model fit was evaluated using the Akaike information criterion (AICc) modified for small sample size (Hurvich and Tsai 1989; Burnham and Anderson 2004). We conservatively considered that a difference in AICc of at least 4 indicated that one model was better
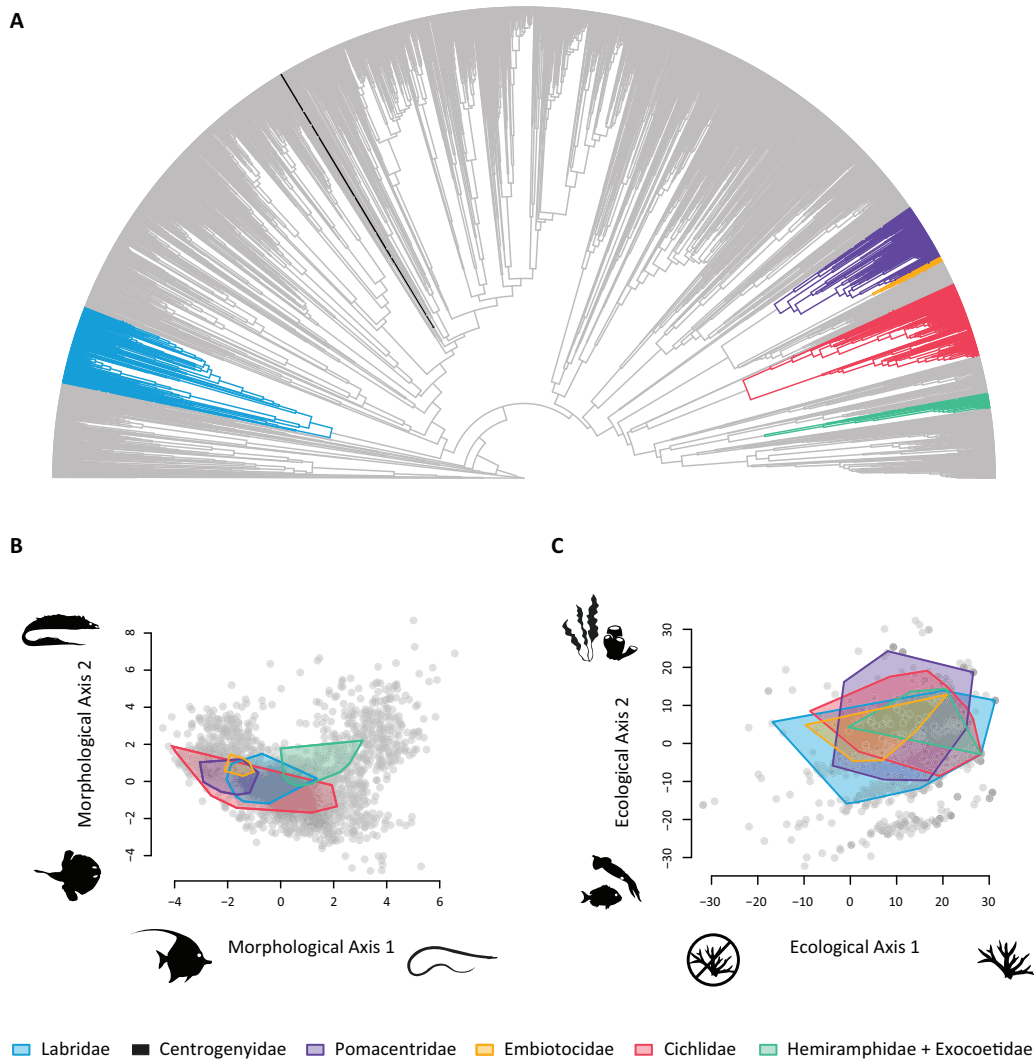
Figure 1. (A) Stochastic character map showing the 6 evolutionary transitions to pharyngognathy among Acanthomorpha (topology pruned from Rabosky et al. 2018). (B) Morphospace occupation of pharyngognathous and non-pharyngognathous taxa along PCs 1 and 2. (C) Ecospace occupation of pharyngognathous and non-pharyngognathous taxa along PCs 1 and 2.

fitting than another. Given that rate variation is expected across the tree, and that this can lead to more complex models being favored over simpler ones, we also performed model fitting and comparison under a "null" scenario. In this null scenario, the 6 pharyngognathous clades were scored as non-pharyngognathous, although 6 other non-pharyngognathous clades of comparable sizes were scored as pharyngognathous. We then fit the single-rate and the two-rate models as described above. Under this null scenario, greater support for the two-rate model would suggest that rate heterogeneity not attributable to pharyngognathy is sufficient to favor the two-rate model.

Given that model-fitting in OUwie is performed in a univariate framework and that the morphological traits are part of a multivariate phenotype, we also compared multivariate phenotypic rates between pharyngognathous and non-pharyngognathous fishes using the *compare.evol.rates* function in the R package geomorph (Adams 2014; Adams et al. 2020). This method estimates the net rate of Brownian phenotypic evolution among groups and generates a ratio between the highest and the lowest of these rates. To determine its statistical significance, the observed rate ratio is then compared with

a null distribution of rate ratios generated by 1,000 random permutations of the tip data on the phylogeny.

The rate comparisons described above were also implemented for each of the pharyngognathous clades and their closest relatives (usually the sister clade but see below for Pomacentridae and Embiotocidae). Each pharyngognathous lineage shared a common evolutionary history with their sister clade prior to divergence, and presumably prior to the evolution of pharyngognathy. Therefore, these sister clades had comparable evolutionary potential at the time of their divergence and comparisons between them are more likely to reveal consistent effects of transitions to pharyngognathy than an analysis of rates across the full dataset. For three pharyngognathous clades, Cichlidae, Labridae, and Exocoetidae+Hemiramphidae, comparisons were made with their respective sister clades. Because Pomacentridae is sister to the clade containing Embiotocidae and non-pharyngognathous lineages, a fourth analysis compared these two pharyngognathous families to the other members of this clade. In other cases where a pharyngognathous family was nested within the sister clade, it was excluded from the analyses. Finally, the sister

clade approach was not used for the Centrogenyidae given that false scorpionfishes are a monospecific family.

Testing for shifts or expansions in morphospace and dietary space

We used ordination to visualize whether pharyngognathy has led to differences in the occupation of morphological and dietary space relative to non-pharyngognathous acanthomorph taxa. To create the morphospace, we performed principal component analysis (PCA) on the correlation matrix of the 8 morphological variables for the full dataset as well as for each of the sister clade pairs. We used non-phylogenetic PCA instead of phylogenetic PCA and focused on the first two principal component (PC) axes, as we expect a key innovation to impact the primary axes of variation. Therefore, when comparing sister clades with and without the innovation, we expect these primary axes to contain the impact of pharyngognathy and to distinguish the two clades, meaning that we wish to retain the variation associated with phylogeny. For comparison, we provide the results from phylogenetic PCAs (Revell 2009) in the Supplementary Material.

We also compared multivariate disparities between pharyngognathous and non-pharyngognathous taxa using the *morphol.disparity* function from the R package geomorph (Adams et al. 2020). Disparity is computed as the sum of the diagonal elements of the group covariance matrices (Zelditch et al. 2012). The statistical significance of the pairwise disparity comparisons was assessed using 1,000 random permutations. Multivariate disparities were calculated on both the log-transformed species averages (i.e., before size-standardization) and on the log-shape ratios.

To determine whether transitions to pharyngognathy have led to differences in dietary niche occupation we performed logistic PCAs on the diet variables using the logisticPCA R package (Landgraf and Lee 2015). We chose logistic PCA over other methods such as NMDS that are commonly used to compare diets (e.g., Abookire et al. 2007; Selleslagh and Amara 2015; Cusa et al. 2019) as preliminary analyses of test datasets revealed it to be the best method for capturing both shifts and expansion in dietary space. For each analysis, we excluded diet variables that contributed information for <1% of the species to ensure the primary axes of variation were not influenced by rare dietary variables. This resulted in different subsets of diet items specific to each logistic PCA analysis. We performed cross validation to determine the value used to approximate the natural parameters from the saturated model and reduced each diet matrix to two dimensions. These analyses were performed on the full dataset and on each of the sister clade pairs.

The morphological and dietary spaces occupied by pharyngognathous and non-pharyngognathous sister clades were quantified by calculating the two-dimensional area of the convex hull encompassing each set of taxa. Convex hulls were calculated using the *chull* function in the R package grDevices (R Core Team 2020), and areas were calculated using the *st_area* function in the R package sf (Pebesma and Bivand 2018). We used the convex hull areas to compute a series of metrics that provide additional information about the patterns of occupation of morphological and dietary space. More specifically, we divided the area of each convex hull by the number of species that it contains [area per species (APS)] to indicate the dispersion of species in morpho- and ecospace. To facilitate comparisons between pharyngognathous clades and their sister, we calculated ratios of convex hull area and APS. Ratios >1 indicate pharyngognathous values are higher than for their sister clade, whereas ratios <1 indicate pharyngognathous values are lower than for their sister clade. We also calculated the proportion of overlap between the convex hulls of pharyngognathous clades and their sister clade. Zero overlap indicates that pharyngognathous taxa occupy an entirely distinct region of morphological or dietary space, whereas overlap of 1 indicates that pharyngognathous taxa are nested entirely within the convex hull of the non-pharyngognathous taxa.

To determine whether patterns of morphological and dietary space occupation between pharyngognathous clades and their sister differ from other family-level clades, we generated a null set of sister clades to compare with the observed metrics. We applied family-level taxonomy according to Fishbase (Froese and Pauly 2020) with some adjustments following Nelson et al. (2016) and extracted from the phylogeny all clades containing a monophyletic, non-monotypic family and its sister that had comparable species richness to the pharyngognathous families and their sister clades. Bounds for species richness, therefore, applied to both the focal family clade and its sister and were based on the minimum ($n = 13$) and maximum ($n = 1,797$) number of species present in either the morphological or dietary space sister clade analyses. We ensured that sister clades appeared only once within the set. Once extracted, the null set of sister clades was matched to the morphological and dietary data and analyzed using the PCA methods described above. The availability of diet data varied for each set of sister clades. Once matched to the diet data, clades with fewer than two species were removed from further analysis, as were clades where the number of species did not exceed the number of dietary variables. Convex hull areas were calculated for each clade and used to compute the metrics and ratios described for the sister clade comparisons above.

To test the prediction that, given the same amount of evolutionary time, transitions to pharyngognathy should lead to an expansion in morphological and/or dietary space, we compared the pharyngognathous clades with a null set of non-pharyngognathous clades with similar crown ages. We set the crown age of each pharyngognathous clade as a target age and extracted all clades that have crown ages within 5 million years of the target age, and more than two species, from the phylogeny. When nested clades were identified, the clade with the crown age closest to the target age was extracted first, then based on the initial extraction other clades within the nested group were extracted when possible to avoid clade-nestedness. Each set of extracted clades was matched to the morphological and diet data and analyzed using non-phylogenetic and logistic PCA methods. Once matched to the diet data, clades were removed following the same criteria as described above, while also removing clades with fewer than three dietary variables. Convex hull areas were calculated for all clades in each set and used to create null distributions of the APS per million years.

Results

Have transitions to pharyngognathy led to increased phenotypic rates?

At the scale of the full acanthomorph dataset, the two-rate model was preferred over the single rate model for most morphological traits except maximum body depth and mouth width, where differences in AICc did not favor one model over the other (Table 1, Figure 2). Wherever the two-rate model was preferred, phenotypic rates were generally higher among the non-pharyngognathous taxa, except for head depth and lower jaw length, which evolved faster in pharyngognathous lineages. When fitting the two models to the null scenario dataset, the two-rate model was preferred for all morphological variables except for maximum fish width (Table 1 and Figure 2), suggesting that factors unrelated to the emergence of

Table 1. Model fitting results for the observed and null scenario datasets comparing a single rate of Brownian evolution across the tree to a two-rate model with rate shifts coinciding with transitions to pharyngognathy

Observed

	Single rate model		Two-rate model			Rate ratio
	AICc	$\sigma2$ (SE)	AICc	$\sigma 2$ (SE)		
				Non-pharyngognathous	Pharyngognathous	
Standard length	−5,055.39	7.89e^{-4} (2.28e^{-2})	**−5,065.48**	*8.14^{-4} (2.49e^{-2})	6.52e^{-4} (5.71e^{-2})	**1.25**
Maximum body depth	−5,299.59	7.40e^{-4} (2.28e^{-2})	−5,297.62	7.41e^{-4} (2.49e^{-2})	7.33e^{-4} (5.72e^{-2})	1.01
Maximum fish width	−5,330.31	7.34e^{-4} (2.28e^{-2})	**−5,402.86**	*7.89e^{-4} (2.48e^{-2})	4.45e^{-4} (5.70e^{-2})	**1.77**
Head depth	−3,588.63	1.15e^{-3} (2.28e^{-2})	**−3,595.22**	1.12e^{-3} (2.49e^{-2})	*1.34e^{-3} (5.71e^{-2})	**1.20**
Lower jaw length	1,794.46	4.67e^{-3} (2.28e^{-2})	**1,725.61**	4.23e^{-3} (2.49e^{-2})	*6.95e^{-3} (5.70e^{-2})	**1.64**
Mouth width	2,791.50	6.04e^{-3} (2.28e^{-2})	2,793.32	6.02e^{-3} (2.49e^{-2})	6.18e^{-3} (5.70e^{-2})	1.03
Min caudal peduncle depth	728.39	3.54e^{-3} (2.28e^{-2})	**328.52**	*4.03e^{-3} (2.48e^{-2})	9.20e^{-4} (5.71e^{-2})	**4.38**
Min caudal peduncle width	4,436.50	9.26e^{-3} (2.28e^{-2})	**4,421.83**	*9.61e^{-3} (2.48e^{-2})	7.40e^{-3} (5.70e^{-2})	**1.30**

Null

	Single rate model		Two-rate model			Rate ratio
	AICc	$\sigma2$ (SE)	AICc	$\sigma 2$ (SE)		
				Non-pharyngognathous	Pharyngognathous	
Standard length	−5,055.39	7.89e^{-4} (2.28e^{-2})	**−5,093.32**	*8.33e^{-4} (2.48e^{-2})	5.50e^{-4} (5.76e^{-2})	**1.52**
Maximum body depth	−5,299.59	7.40e^{-4} (2.28e^{-2})	**−5,304.60**	*7.59e^{-4} (2.48e^{-2})	6.40e^{-4} (5.76e^{-2})	**1.18**
Maximum fish width	−5,330.31	7.34e^{-4} (2.28e^{-2})	−5,329.13	7.41e^{-4} (2.48e^{-2})	7.00e^{-4} (5.75e^{-2})	1.06
Head depth	−3,588.63	1.15e^{-3} (2.28e^{-2})	**−3,628.70**	*1.22e^{-3} (2.48e^{-2})	7.96e^{-4} (5.75e^{-2})	**1.53**
Lower jaw length	1,794.46	4.67e^{-3} (2.28e^{-2})	**1,573.62**	*5.20e^{-3} (2.48e^{-2})	1.81e^{-3} (5.75e^{-2})	**2.88**
Mouth width	2,791.50	6.04e^{-3} (2.28e^{-2})	**2,784.04**	*6.22e^{-3} (2.48e^{-2})	5.11e^{-3} (5.74e^{-2})	**1.22**
Min caudal peduncle depth	728.39	3.54e^{-3} (2.28e^{-2})	**541.96**	*3.92e^{-3} (2.48e^{-2})	1.50e^{-3} (5.76e^{-2})	**2.62**
Min caudal peduncle width	4,436.50	9.26e^{-3} (2.28e^{-2})	**4,357.08**	*9.97e^{-3} (2.48e^{-2})	5.44e^{-3} (5.75e^{-2})	**1.83**

Bold fonts are used to identify models that are substantially supported as having a better fit based on a ΔAICc >4. When the two-rate model is preferred, an asterisk is used to indicate which of the non-pharyngognathous or pharyngognathous taxa exhibit higher phenotypic rates. Rate ratios correspond to the ratio between the highest and lowest phenotypic rates estimated under the two-rate model.

pharyngognathy influence rates of phenotypic evolution across acanthomorphs.

We computed ratios between the highest and lowest phenotypic rates estimated under the two-rate model for each morphological variable for both the observed dataset and the null scenario dataset (Table 1). If transitions to pharyngognathy have led to shifts in phenotypic rates, we would expect rate ratios for the observed dataset to be above the bounds of those obtained for the null dataset. Only for minimum caudal peduncle depth did we obtain an observed rate ratio above the rate ratios obtained from the null

dataset—however, rates were estimated to be higher among non-pharyngognathous taxa.

The results from comparisons between pharyngognathous clades and their closest relatives varied (Figure 2, Supplementary Tables S2–5)—the two-rate model received strong support for a minimum of 2 and a maximum of all 8 morphological traits in each sister clade analysis. None of the morphological traits received consistently strong support for increased rates in pharyngognathous taxa across all four analyses. However, some consistent trends appeared for the Labridae and Cichlidae. The two-rate model was preferred for all
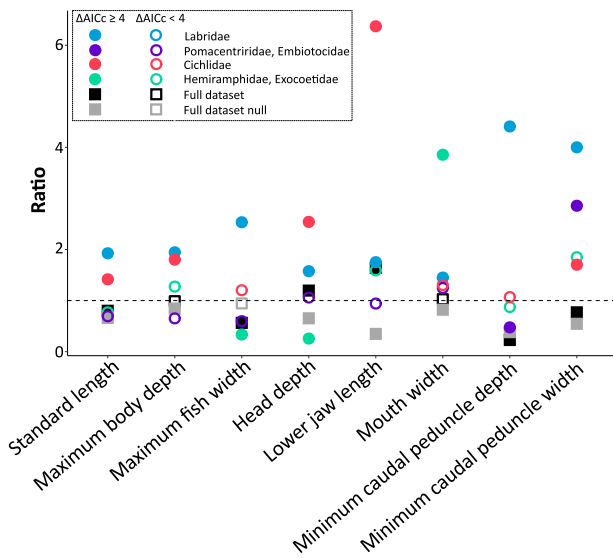
Figure 2. Phenotypic rate ratios between pharyngognathous and non-pharyngognathous clades for each sister clade comparison and the full and null datasets. Ratios were calculated as pharyngognathous rate/non-pharyngognathous rate; ratios >1 (dashed line) indicate that pharyngognathous taxa have relatively higher rates.

comparisons between labrids and their sister clade, phenotypic rates were higher in labrids relative to their sister clade, and caudal peduncle traits displayed the highest rate ratios (Figure 2; Supplementary Table S2). In Cichlidae and their sister clade, the two-rate model was preferred for 5 of the 8 morphological traits, and phenotypic rates were higher in cichlids compared with their sister clade (Figure 2, Supplementary Table S4). Finally, across all comparisons, the highest rate ratio was obtained for lower jaw length in Cichlidae, with higher phenotypic rates in cichlids relative to their sister clade. The results from the comparisons of multivariate rates in geomorph were not entirely consistent with the univariate results from OUwie (Table 2). The greatest inconsistency was generated in Labridae, where the multivariate rate estimated for labrids was less than half that of their sister clade, whereas for univariate traits higher rates were estimated within Labridae.

Have transitions to pharyngognathy led to shifts or expansions in morphological and dietary space?

Across acanthomorphs, all pharyngognathous clades fell within the morphological and ecological space occupied by non-pharyngognathous species (Figure 1B and C). Body elongation, contrasting body depth and length, is the dominant factor along the first principal component axis (PC1) and dorsoventral compression, contrasting body width and length, is the dominant factor along PC 2 (Figure 1B; Supplementary Table S6). In the dietary space, corallivorous species are contrasted with generalists that do not consume coral along the first ecological axis, and species that hunt fishes and cephalopods are contrasted with those that graze on benthic prey items along the second ecological axis (Figure 1C; Supplementary Table S8). Within the context of acanthomorphs, there were no consistent shifts toward or away from particular ecologies or morphologies among pharyngognathous clades.

Relative to their respective sister clades, the magnitude of shifts in morphospace and dietary space varied widely for each pharyngognathous lineage: all pharyngognathous lineages, except for the Pomacentridae+Embiotocidae morphospace, shared some amount of space with their sister clade (Figure 3, Tables 3 and 4 overlap). The null morpho- and dietary spaces also returned the full range of observed overlap values (Tables 3 and 4). In general, pharyngognathous clades shifted toward deeper-bodied morphologies, with deeper heads and caudal peduncles, shorter standard lengths, and lower jaw lengths relative to their sister clades (Figure 3 and Supplementary Table S6). These common shifts in morphology correspond to the occupation of novel areas of dietary space that reflect the consumption of a greater diversity of prey items. The exception to both of these common patterns in morpho- and ecospace is the Labridae, who have not shifted in morphospace or expanded their dietary breadth beyond that of their sister clade.

Across all sister clade comparisons, there was an increase in the area of morphological and dietary space occupied with increasing species richness. Despite this general trend, all pharyngognathous clades occupied smaller areas of morphospace and larger areas of dietary space (except Labridae) relative to their sister clades, regardless of species richness (Figure 3, Tables 3 and 4 area ratios). Similarly, morphological disparities were significantly higher in non-pharyngognathous sister clades except in Beloniformes (Exocoetidae + Hemiramphidae) (see Supplementary Table S9). The area of morphospace occupied by the Labridae relative to their sister clade was lower than any of the area ratios obtained from the null set of sister clades. All other morphological and dietary area ratios were within the bounds of those calculated from the null clades.

After accounting for the species richness of each clade, pharyngognathous species were more clustered in morphospace but more dispersed in dietary space (except Pomacentridae+Embiotocidae) than the non-pharyngognathous species in their sister clades (Figure 3, Tables 3 and 4 APS ratios). The Pomacentridae+Embiotocidae were more clustered in morphospace relative to their sister clade than any of the null comparisons, although their clustering in dietary space was within the bounds of the null. Both the Labridae and the Exocoetidae+ Hemiramphidae were more dispersed in dietary space relative to their sister clades than any of the null comparisons.

Crown ages of pharyngognathous clades ranged from 22.44 to 79.89 million years (Supplementary Table S10). Average rates of morphological and dietary space occupation per species were toward the low end of the range of values recorded from each of their respective null sets of clades (Figure 4), indicating transitions to pharyngognathy may actually slow the rate of morphological and dietary space exploration. This is especially apparent for the Pomacentridae and Embiotocidae whose rates of expansion in morphological (Pomacentridae and Embiotocidae) and dietary (Pomacentridae) space occupation per species were lower than the values recorded from their respective null sets of clades (Figure 4G–I). An APS value for Embiotocidae could not be calculated from the ecological data because the number of species with data available did not exceed the number of dietary variables; therefore, the logistic PCA could not be performed.

Discussion

Pharyngognathy is widely cited as a classic example of a key innovation (Liem 1980; Wainwright et al. 2012; Wainwright and Longo 2017) and was originally described as such based on the exceptional diversity of East-African cichlids (Liem 1973; Liem 1980; Kaufman and Liem 1982). Later, as additional pharyngognathous families were discovered, several comparative studies have looked at the influence of this putative key innovation on lineage diversification rates, yielding mixed results (Alfaro et al. 2009; McGee et al. 2015).

Table 2. Estimates of multivariate net rates of Brownian evolution and rate ratios between the non-pharyngognathous and pharyngognathous taxa

Focal clade	Multivariate rate estimates		Rate ratio and statistical significance	
	Non-pharyngognathous	Pharyngognathous	Ratio	P-value
Acanthomorpha	$3.42e^{-3}$	$3.07e^{-3}$	1.12	0.356
Labridae	$*4.20e^{-3}$	$1.81e^{-3}$	**2.33**	**0.001**
Pomacentridae+Embiotocidae	$1.27e^{-3}$	$1.59e^{-3}$	1.26	0.4
Cichlidae	$2.41e^{-3}$	$*5.10e^{-3}$	**2.12**	**0.002**
Hemiramphidae+Exocoetidae	$3.13e^{-3}$	$5.48e^{-3}$	1.75	0.125

Where the focal clades are pharyngognathous families, the rate comparisons are between those families and their sister clades. Bold fonts are used to indicate statistical significance of the rate ratios based on 1,000 permutations of the tip data. When the rate ratio is significant, an asterisk is used to indicate which of the non-pharyngognathous or pharyngognathous taxa exhibit higher multivariate phenotypic rates.

We focus on the morphological and ecological consequences and find a similar lack of consistency regarding how pharyngognathy affects phenotypic rates, but more consistent patterns of morphospace and dietary space occupation across lineages.

Key innovations are expected to facilitate access to new areas of the adaptive landscape (Simpson 1944; Simpson 1953), leading to the prediction that lineages that possess the trait will diversify more rapidly compared with lineages that do not. Contrary to our expectation, at the scale of the Acanthomorpha, we did not find strong support for increased rates of phenotypic evolution associated with pharyngognathy. Rate heterogeneity across the Acanthomorpha may partly explain why the two-rate model was generally better-fitting for both the observed and the null datasets (Rabosky and Goldberg 2015; Beaulieu and O'Meara 2016; Caetano et al. 2018; May and Moore 2020). As for the generally higher rates obtained for taxa lacking the key innovation, it is important to note that the pharyngognathous taxa comprise only 7 families and are represented in our dataset by 619 species, compared with the 251 families and 3,234 species of non-pharyngognathous taxa, which means the latter are both substantially more speciose and taxonomically diverse; these two features imply that non-pharyngognathous lineages likely exhibit high rate heterogeneity. Given this limitation, the macroevolutionary consequences of pharyngognathy may be more readily apparent when comparing pharyngognathous lineages with their closest relatives lacking the innovation (Mitter et al. 1988; Sanderson and Donoghue 1996; Bond and Opell 1998). These sister clade comparisons can partially control for the wide array of processes, other than those directly related to pharyngognathy, that can influence phenotypic diversification. However, results of the rate comparisons between pharyngognathous lineages and their non-pharyngognathous sister clades were inconsistent, suggesting that lineage-specific effects play an important role and possibly obscure any generalized signature of pharyngognathy on rates of phenotypic evolution.

We found substantial support for increased rates across all traits in Labridae, and in 5 of the 8 morphological traits in Cichlidae. Although cichlids and labrids are the two largest of our pharyngognathous clades, it is unlikely that sampling bias is responsible for these higher rate estimates given that the sister clades to Labridae and Cichlidae are, in both cases, more species-rich and more taxonomically diverse (i.e., number of families). One possible explanation for these increased rates in labrids and cichlids may be that transitions to pharyngognathy can be particularly advantageous when combined with some additional extrinsic or intrinsic factors. For example, an extrinsic factor that may have promoted African cichlid diversification is the absence of competition from other ray-finned fish lineages in Lakes Victoria and Malawi, where the 2

largest radiations occurred (McGee et al. 2015). Labrids have radiated in reef habitats, which are well known for their exceptional diversity and high productivity (Talbot 1965; Risk 1972; Luckhurst and Luckhurst 1978; Bellwood and Wainwright 2002; Alfaro et al. 2007). Pomacentrids also diversified on reefs yet they did not show a pattern of increased phenotypic rates, suggesting that the ecological opportunities offered on reefs alone are not sufficient to explain the increased rates in labrids. Potentially, additional intrinsic factors such as the evolution of the pharyngeal mill, which is thought to further increase the efficiency of processing durophagous dietary items (Gobalet 1989; Evans et al. 2019), or the intramandibular jaw joint (IMJ) found in a subset of parrotfishes (Konow et al. 2008) and thought to improve their ability to scrape corals, interacted with pharyngognathy to drive diversification. Studies have identified increased rates of morphological evolution of the lower pharyngeal jaw in parrotfishes relative to wrasses (Evans et al. 2019) and in the oral jaws of parrotfishes that possess the IMJ relative to other parrotfishes that do not possess the joint (Price et al. 2010). It is therefore conceivable that some of the morphological traits that were considered in this study have also experienced increased evolutionary rates following the evolution of these additional jaw modifications in pharyngognathous labrids.

It may seem surprising that when analyzed in a multivariate framework, the rate estimate is lower for labrids relative to their sister clade when individually, all univariate traits are faster in labrids. This may be partly due to the taxonomic diversity of the sister clade to Labridae, which contains taxa that are well known for being morphologically disparate [e.g., Tetraodontiformes (Winterbottom 1974; Tyler 1980), Gasterosteiformes (Orr 1995)]. Therefore, the Labridae may be more constrained in their multivariate phenotypes because they are a single family, compared with their sister clade, which includes 123 acanthomorph families. In other words, perhaps in labrids, most of the traits are co-evolving, and even if they are doing so at a fast pace, they are following similar evolutionary trajectories across species. Consequently, less multivariate disparity is generated than in the sister clade, where the co-evolution between traits is more varied due to their taxonomic diversity.

Key innovations are also expected to generate shifts and/or expansions in morphological and ecological space (Heard and Hauser 1995; Bond and Opell 1998; Rabosky 2017). As with the rate analyses, at the scale of acanthomorphs, we did not find strong evidence for shifts or expansions in either morphological or dietary space (Figure 1B and C). However, shifts were apparent when comparing pharyngognathous lineages with their respective sister clades, except Labridae, which are entirely nested within the morphospace and ecospace occupied by their sister clade (Figure 3). As previously
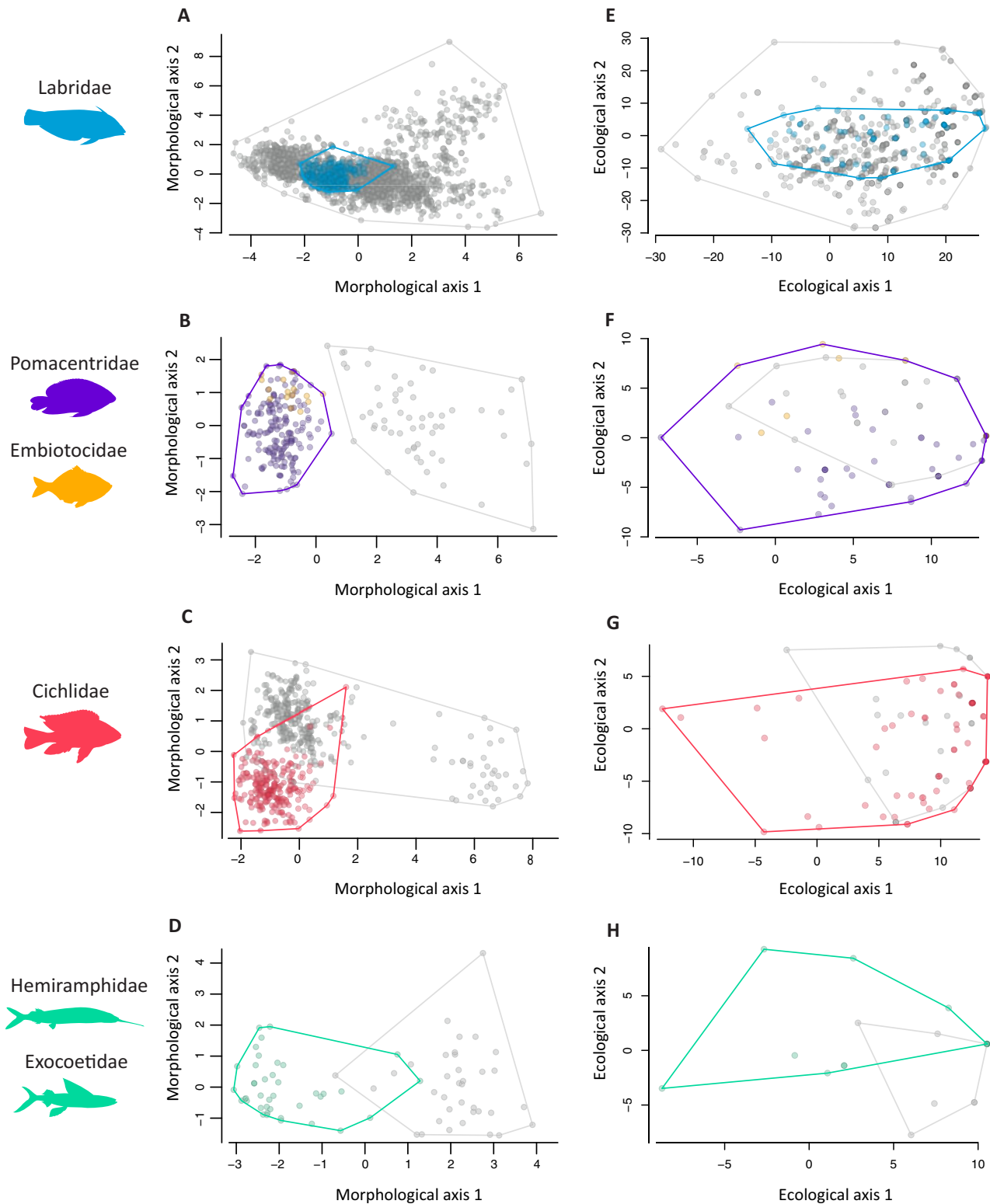
Figure 3. Morpho- and ecospace occupation of pharyngognathous and non-pharyngognathous taxa for each of the sister clade comparisons.

mentioned, the clade sister to the Labridae is more taxonomically diverse with greater species richness—it contains 8 times more species than the Labridae. Therefore, the labrid transition to pharyngognathy either had less of an impact on the lineage's morphology and dietary ecology relative to their sister lineage, which is entirely

probable given its size and diversity, or the impact is masked by lack of resolution along the backbone of the phylogeny lumping the true sister clade inside a much larger clade.

The clustering of pharyngognathous clades in morphospace and their dispersion in dietary space relative to their sister clades suggest

Table 3. Patterns of morphological space occupation of pharyngognathous clades relative to their sister clade and for the null set of sister clade comparisons

Morphological space

	Sister clade			Pharyngognathous clade					
	Convex hull area	No. spp	APS	Convex hull area	No. spp	APS	Area ratio	APS ratio	Overlap
Labridae	91.40	1,797	0.05	6.69	209	0.03	0.07	0.60	1.000
Pomacentridae + Embiotocidae	24.57	58	0.42	9.09	166	0.05	0.37	0.12	0.000
Cichlidae	31.82	284	0.11	11.98	203	0.06	0.38	0.50	0.541
Hemiramphidae + Excocoetidae	15.01	36	0.42	9.76	40	0.24	0.65	0.57	0.221

Morphological space null

	Sister clade			Focal family clade					
Mullidae	16.56	17	0.97	3.79	26	0.15	0.23	0.15	0.00
Pomacanthidae	22.95	118	0.19	8.85	41	0.22	0.39	1.11	0.00
Fundulidae	6.04	18	0.34	12.66	28	0.45	2.09	1.35	0.01
Monacanthidae	9.24	31	0.30	30.94	43	0.72	3.35	2.41	0.10
Sparidae	13.77	23	0.60	29.80	60	0.50	2.16	0.83	0.29
Blenniidae	26.56	16	1.77	9.14	28	0.33	0.34	0.18	0.34
Chaetodontidae	22.49	22	1.02	23.99	81	0.30	1.07	0.29	0.36
Rivulidae	10.80	16	0.67	24.02	20	1.26	2.22	1.87	0.36
Malacanthidae	34.84	107	0.33	11.62	15	0.77	0.33	2.38	0.58
Holocentridae	21.21	32	0.66	6.30	43	0.15	0.30	0.22	0.62
Sciaenidae	34.21	122	0.28	16.51	64	0.26	0.48	0.92	0.94
Lethrinidae	43.33	83	0.52	11.78	24	0.49	0.27	0.94	0.98

Ratios are calculated as the pharyngognathous value divided by the non-pharyngognathous value. Overlap is the proportion of the pharyngognathous area shared with the area occupied by its sister clade.

Table 4. Patterns of dietary space occupation of pharyngognathous clades relative to their sister clade and to the null set of sister clades

Dietary space

	Sister clade			Pharyngognathous clade					
	Convex hull area	No. spp	APS	Convex hull area	No. spp	APS	Area ratio	APS ratio	Overlap
Labridae	3,145.21	893	3.52	824.50	104	7.93	0.26	2.25	1.000
Pomacentridae + Embiotocidae	139.85	30	4.66	270.57	122	2.22	1.93	0.48	0.517
Cichlidae	180.10	66	2.73	291.27	79	3.69	1.62	1.35	0.474
Hemiramphidae + Excocoetidae	45.73	19	2.41	138.24	13	10.63	3.02	4.42	0.101

Dietary space null

	Sister clade			Focal family clade					
Blenniidae	22.89	6	3.82	117.38	17	6.90	5.13	1.81	0.000
Sparidae	46.33	9	5.15	384.93	47	8.19	8.31	1.59	0.108
Pomacanthidae	233.03	78	2.99	92.95	36	2.58	0.40	0.86	0.280
Chaetodontidae	290.28	12	24.19	146.10	52	2.81	0.50	0.12	0.296
Monacanthidae	333.12	21	15.86	205.25	18	11.40	0.62	0.72	0.831
Holocentridae	183.36	11	16.67	114.47	33	3.47	0.62	0.21	0.859
Malacanthidae	379.93	71	5.35	1.07	3	0.36	0.00	0.07	1.000
Sciaenidae	378.18	74	5.11	75.74	47	1.61	0.20	0.32	1.000
Lethrinidae	380.43	56	6.79	97.12	15	6.47	0.26	0.95	1.000
Mullidae	NA	2	NA	122.17	17	7.187	NA	NA	NA
Fundulidae	NA	NA	NA	NA	NA	NA	NA	NA	NA
Rivulidae	NA	NA	NA	NA	NA	NA	NA	NA	NA

Ratios are calculated as the pharyngognathous value divided by the non-pharyngognathous value. Overlap is the proportion of the pharyngognathous area shared with the area occupied by its sister clade.
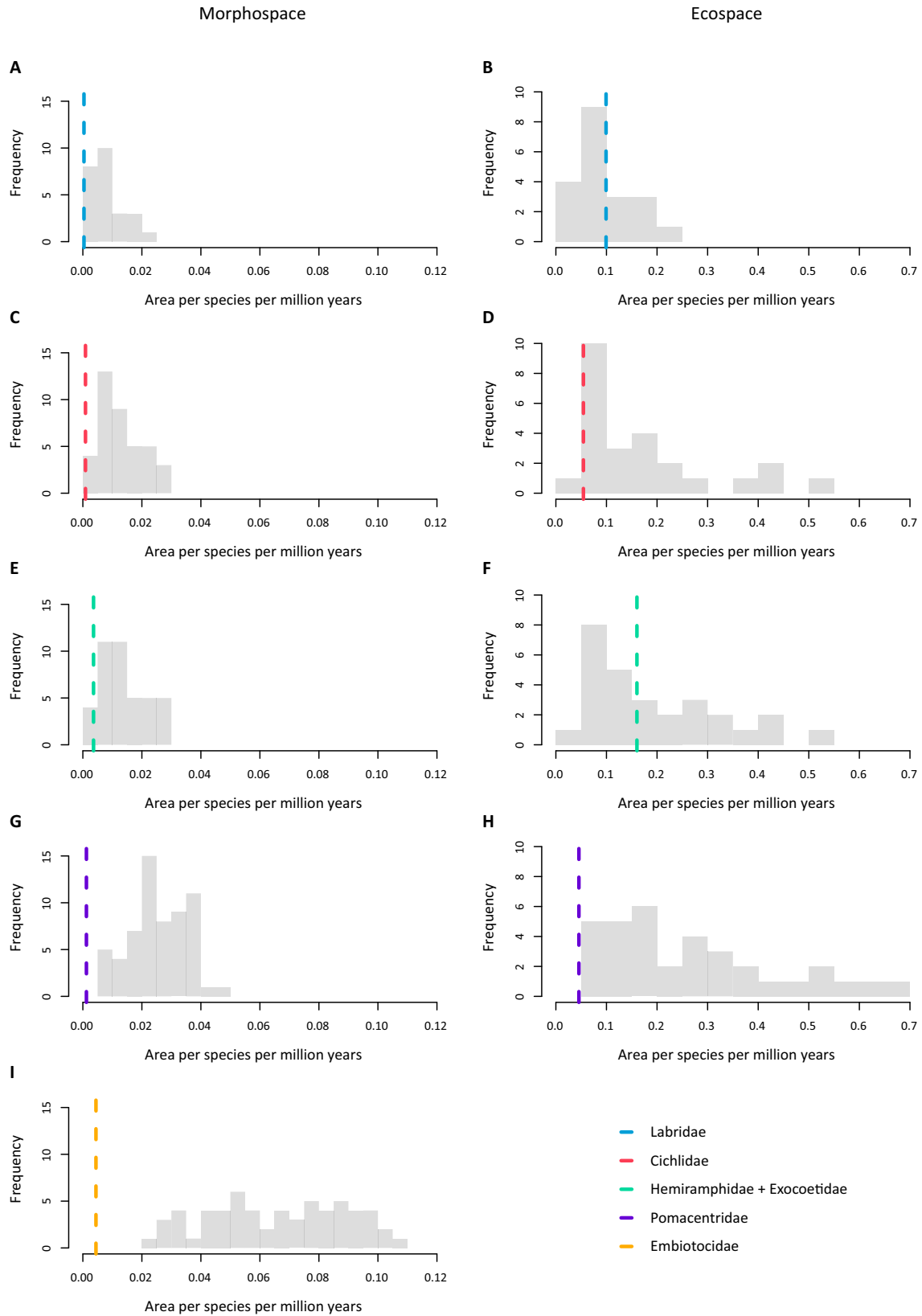
Morphospace

Ecospace

Figure 4. Histograms comparing the APS per million years for each pharyngognathous clade (colored dashed lines) with the distribution of values calculated from the null set of clades with comparable crown ages. Pharyngognathous lineages have been ordered from top to bottom by clade age.

that shifts to a novel morphology impose constraints on subsequent morphological evolution, but these smaller morphological changes likely lift ecological constraints allowing pharyngognathous species to increase the diversity of their diets. This agrees with (and potentially expands on) the classic predictions of key innovation theory (Simpson 1944, 1953; see also Rabosky 2017). However, another possible explanation for the contrasting patterns observed in morphospace relative to dietary space is that the latter may be more directly related to the expected functional consequences of pharyngognathy, that is increased effectiveness in processing hard and tough prey, and increased versatility in prey-capture (Burress et al. 2020). In comparison, excepting mouth width and lower jaw length, most of our morphological traits capture information about overall body shape. Perhaps, a dataset focusing more on cranial/oral anatomy would provide additional insights into the influence of pharyngognathy on phenotypic evolution.

Although assessing expansion in morphological and ecological space using a sister clade approach reduces the effects of clade age, there can still be considerable differences between the crown age of the focal clade and that of its sister clade. As we have no way of knowing with confidence exactly when pharyngognathy evolved along the stem of each pharyngognathous clade, we performed comparisons with clades that have similar crown ages to investigate rates of expansion in morphological and dietary space. The results of these comparisons did not support the predicted expansion in morphological or dietary space in pharyngognathous lineages. Instead, these results suggest some possible constraints on the rate of expansion, particularly for the pomacentrid dietary space. This is consistent with findings from previous empirical studies, which have shown that evolutionary innovations do not always lead to an expansion of the niche but that they can instead constrain it (Holliday and Steppan 2004; Ord et al. 2020). However, this metric does not contain information about novel space occupation, it simply quantifies the average area of space occupied per species per million years. Therefore, pharyngognathous taxa do not expand in morphospace or ecospace faster than other, more distantly related clades with comparable ages. Furthermore, we cannot confidently infer dietary constraints, as we were not able to perform the ordination used to construct the dietary space on clades with very low diet diversity.

During the development of this study, we have faced several challenges when testing key innovation hypotheses. One of the current analytical challenges is rate heterogeneity across large phylogenies (Rabosky et al. 2013; Chira and Thomas 2016) as it can lead to more complex models being favored because they allow for some rate variation (Rabosky and Goldberg 2015; Beaulieu and O'Meara 2016; Caetano et al. 2018; May and Moore 2020). We therefore need null models that allow for rate variation not associated with the key innovation (Beaulieu and O'Meara 2016). The development of trait diversification models that take into account rate heterogeneity has lagged behind those for lineage diversification (Rabosky and Goldberg 2015; Beaulieu and O'Meara 2016). We chose to investigate the potential impact of rate heterogeneity by generating a null distribution of pharyngognathy on the phylogeny, identifying clades that had similar sizes to the true pharyngognathous lineages and rerunning all analyses with these "dummy" pharyngognathous variables to see if two-rate models were preferred, and if so, quantify the difference in rate between the dummy pharyngognathous and non-pharyngognathous clades. However, we were limited in the number of clades that matched the largest pharyngognathous clades (i.e., Labridae and Cichlidae) in size, leaving us with a single null distribution, which is far from ideal. Moreover, for the sister clade comparisons, even with a fairly large dataset/phylogeny, we could not control simultaneously for both the size of the dummy pharyngognathous clades and the size of their sister clades, resulting in comparisons that differed in taxonomic scope between the observed and null datasets. Recent methodological developments avoid these issues by either incorporating rate heterogeneity into their state-dependent models of diversification (May and Moore 2020) or using Hidden-Rate Markov Models (Boyko and Beaulieu 2020), and therefore look particularly promising for future key innovation studies.

An additional difficulty we encountered when generating null distributions was that the most appropriate null depended on the prediction being tested. For instance, the sister clade comparisons were most appropriate for testing shifts in morphological or dietary space occupation, yet a crown age approach was most appropriate for testing rates of expansion in those spaces. In both cases, additional analytical constraints led to a very reduced number of clades that could be used to generate the null distribution. Indeed, the ordination approach that was used to construct the morphological and dietary spaces required the number of species in each clade to exceed the number of variables in the dataset. Therefore, we could not include clades with highly diverse diets expressed by a handful of species, nor could we include clades with very low dietary diversity as it rendered it impossible to construct a convex hull or quantify the subsequent metrics based on that area.

Key innovations may allow shifts in evolutionary rates (e.g., Garcia-Porta and Ord 2013; Maia et al. 2013), shifts and/or expansions in morphological and ecological space (e.g., Bond and Opell 1998), and may even constrain phenotypic evolution (e.g., Holliday and Steppan 2004; Ord et al. 2020). Yet most empirical studies thus far have focused on lineage diversification or only one of these phenotypic consequences at a time. While pharyngognathy is a widely cited, classic example of a key innovation, our results only support some but not all of the predicted macroevolutionary consequences of a key innovation and rarely were the outcomes exceptional compared with our null models. We therefore suggest that it may be time to reconsider the expected evolutionary impacts of key innovations. First, should key innovations promote lineage, morphological and ecological diversification simultaneously, or is one of these sufficient? Our results show that having multiple independent evolutionary origins of a key innovation, a necessary condition for testing hypotheses using a phylogenetic comparative framework, may also have unintended consequences for our ability to identify the signature of key innovations. Pharyngognathy evolved in discrete clades with a large amount of evolutionary history between them, which increases the opportunity for the evolution of lineage-specific effects that may obscure the expected patterns of morphological and ecological diversification. In this sense, the multiple evolutionary origins of pharyngognathy are both an advantage and a disadvantage, as these lineages have distinct sets of traits and experience different ecological circumstances that may complicate finding a consistent signature of the putative key innovation. Second, should a key innovation generate macroevolutionary signatures that are significantly different from, or outside the bounds of suitable null distributions? Our results show some consistency in patterns of morphospace and ecospace occupation, however, most were not exceptional when compared with the nulls. Is it reasonable to expect that the evolution of a single key innovation would generate macroevolutionary patterns that standout against a backdrop of all other evolution in that lineage? It is likely that, given sufficient time, most lineages will eventually access previously unavailable adaptive

zones, either through the evolution of a novel phenotype or another mechanism. These events also have the potential to generate identifiable macroevolutionary signatures. Researchers interested in a purported key innovation should carefully consider its context with this in mind. One promising approach might be to envisage using a more holistic framework to test key innovation hypotheses, such as the phylogenetic natural history approach described in Uyeda et al. (2018) that may help disentangle lineage-specific effects from those that can be traced back to the trait of interest. We also suggest that focusing on the most direct consequences of the innovation will help provide both compelling examples of how innovations shape diversification as well as instructive counter-examples.

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Authors' Contributions

O.L., J.R.H., L.R.V.A., B.C., D.S.A., K.Z., and S.A.P. conceived the study, analyzed the data and drafted the manuscript with input from S.T.F. and P.C.W; O.L., S.T.F., K.Z., P.C.W., and S.A.P. collected the morphometric data.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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