

Modular Diversification of the Locomotor System in Damselfishes (Pomacentridae)

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ABSTRACT As fish move and interact with their aquatic environment by swimming, small morphological variations of the locomotor system can have profound implications on fitness. Damselfishes (Pomacentridae) have inhabited coral reef ecosystems for more than 50 million years. As such, habitat preferences and behavior could significantly constrain the morphology and evolvability of the locomotor system. To test this hypothesis, we used phylogenetic comparative methods on morphometric, ecological and behavioral data. While body elongation represented the primary source of variation in the locomotor system of damselfishes, results also showed a diverse suite of morphological combinations between extreme morphologies. Results show clear associations between behavior, habitat preferences, and morphology, suggesting ecological constraints on shape diversification of the locomotor system. In addition, results indicate that the three modules of the locomotor system are weakly correlated, resulting in versatile and independent characters. These results suggest that Pomacentridae is shape may result from the interaction between (1) integrated parts of morphological variation that maintain overall swimming ability and (2) relatively independent parts of the morphology that facilitate adaptation and diversification. *J. Morphol.* 000:000–000, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: modularity; integration; locomotion; geometric morphometrics; reef fish; ecomorphology; evolutionary morphology

INTRODUCTION

Considerable morpho-functional diversity in fishes is seen in the structures of the locomotor system because density-related inertial forces in aquatic systems have high impacts on swimming performance (Videler, 1993). Because swimming is the primary way that fish interact and move through their environment (Fulton, 2010), swimming performance is a major determinant of (1) individual survival, (2) growth, and (3) lifetime reproductive fitness (Webb, 1994; Plaut, 2001; Blake, 2004). As such, small variations in swimming abilities within and among species can have

profound impacts on these three key life history parameters (Koch and Wieser, 1983; Boisclair and Sirois, 1993), making swimming performance a primary target for natural selection (Huey and Stevenson, 1979; Hertz et al., 1988).

Many studies have examined the role of body and fin shape on swimming performance in teleosts (Webb, 1982; Bushnell and Moore, 1991; Lighthill, 1993; Gibb et al., 1994; Gibb et al., 1999; Drucker and Lauder, 2001, 2005; Wainwright et al., 2002; Fulton et al., 2005; Fulton, 2007; Standen, 2008; Aguilar-Medrano et al., 2013). Fewer studies have examined the coordination of the locomotor and feeding system (Rice and Westneat, 2006; Higham, 2007), and only one study has explicitly tested the correlated evolution of teleost feeding and locomotor systems within a single clade (i.e., labrids; Collar et al., 2008). As such, it is unclear how morphological variation within a single taxonomic group may be correlated to niche partitioning via swimming performance during evolution.

Pomacentridae (damselfishes) is a speciose family of 394 species of marine fish (Eschmeyer, 2014) that has inhabited reef ecosystems for more than 50 million years (Bellwood, 1996; Bellwood and Sorbini, 1996). The vast majority of damselfish inhabit tropical coral reefs, although some species live in rocky reefs or kelp forests in temperate coastal waters. Due to their wide range of

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behaviors and habitat preferences in structurally complex environments, damselfishes are an excellent model to test the role of the locomotor system shape on niche partitioning and diversification.

The majority of ecomorphological studies on damselfishes have focused on the relationship between morphology and diet (Emery, 1973; Frédérick et al., 2008; Cooper and Westneat, 2009; Aguilar-Medrano et al., 2011; Frédérick et al., 2013). Only two key studies analyzed the ecomorphological variation of swimming performance (Fulton, 2007; Aguilar-Medrano et al., 2013). In this study, we analyze the morphology of the locomotor system of damselfishes from the perspective of modularity. Modularity refers to semiautonomous units (modules) that comprise higher organisms (Simon, 1962; Wagner and Altenberg, 1996). Modules are assemblages of parts that are integrated internally by strong interactions, but are relatively independent of one another by few or weak interactions (Wagner and Altenberg, 1996; Klingenberg, 2005). We focus on modules because of their possible role as “building blocks” of phenotypic adaptation (Wagner, 1995).

Functional modularity is an important determinant of evolutionary diversification because it provides a link between the modular structure of morphological traits and selection on performance in organismal functions (Klingenberg, 2008). Hence, in an evolutionary context, a module is a set of morphological characters that (1) collectively serve a common functional role, (2) are tightly integrated, and (3) are relatively independent from other modules (Mitteroecker and Bookstein, 2007).

Here, we focus on three morphological modules that play different roles in swimming fitness: *module 1*, cephalic region, associated with drag reduction and lift forces (Bushnell and Moore, 1991; Lighthill, 1993; Larouche et al., 2015); *module 2*, trunk, dorsal, pelvic, and anal fins, associated with stability and thrust (Harris, 1938; Drucker and Lauder, 2001, 2005; Fulton, 2007; Standen, 2008); and *module 3*, caudal peduncle, associated with acceleration and thrust (Webb, 1982; Gibb et al., 1999; Fig. 1). We study the variation along these morpho-functional axes in an evolutionary context, focusing on the relationship between habitat preferences and behavior to address three questions: (1) how the morphological modules vary within Pomacentridae, (2) do these morpho-functional modules evolve individually or as an integrated system?, and (3) do behavior and habitat preferences impact the morphological variation of each module?

MATERIAL AND METHODS

Morphological Data

We conducted morphometric analyses on 739 lateral-view photographs of adult damselfish, representing 205 species from the time-calibrated phylogeny of Frédérick et al. (2013). These samples represent 27 of 29 genera; however, the two missing genera, *Amblypomacentrus* and *Altrichthys*, only contain two

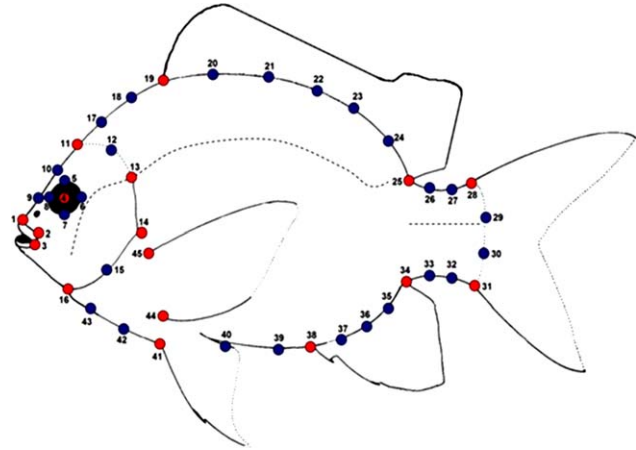


Fig. 1. Landmarks (LMs, red dots) and semilandmarks (SLMs, blue dots) used to analyze the locomotor system of damselfishes. A: *module 1*, cephalic region (CR; LMs & SLMs 1–18); *module 2*, trunk (LMs & SLMs 19 to 25 and 34 to 45); *module 3*, caudal peduncle (CP; LMs & SLMs 26 to 33).

species, are very similar in shape to *Pomacentrus* and *Chrysiptera* (Allen, 1991) and are recently diverged from these genera (Frédérick et al., 2013). As such, the absence of these genera should not impact the results of the study. Photographs were largely obtained directly from accessioned museum specimens, with additional photographs from online resources such as MorphBank (www.morphbank.net), FishBase (Froese and Pauly, 2014) and John E. Randall’s Fish Photos (pbs.bishopmuseum.org/images/JER). The complete list of species included in this study, their sources and accession numbers are available as supplementary online material 1.

We conducted landmark (LM)-based geometric morphometric analyses (Bookstein, 1991; Rohlf and Marcus, 1993; Rohlf, 1999; Zelditch et al., 2004), recording x , y coordinates of LMs and semilandmarks (SLMs) using TpsDig, version 2.17 (Rohlf, 2014). SLMs were identified using a slider file made in TpsUtil (Rohlf, 2014). First, we studied the whole body shape variation using a configuration made of 17 LMs and 28 SLMs. Then, the overall body was divided into three modules based on functional analyses: *module 1*: cephalic region delimited by eight LMs and 10 SLMs; *module 2*: trunk delimited by seven LMs and 14 SLMs, and *module 3*: caudal peduncle delimited by two LMs and six SLMs (Fig. 1). For every data set, superimposition of LM configurations was achieved using Generalized Procrustes Analysis (Rohlf and Slice, 1990). We calculated Partial Warp scores, and we performed a Principal Components Analysis (PCA) of shape variables (also called Relative Warps analysis, RWs) using TpsRelw, version 1.54 (Rohlf, 2014). PCA was used to find hypothetical variables (components) that account for much of the variance in the morphological data (Davis, 1986). Principal components scores were used as descriptors of shape variation (Bookstein, 1991; Rohlf, 1993). Deformation grids using the thin-plate spline algorithm were used to visualize the patterns of shape variations along PC axes (Bookstein, 1991; Rohlf, 1993).

We tested the relevance of modules defined *a priori* based on functional considerations using Escoufier’s RV-coefficient and the multi-set RV-coefficient (Klingenberg, 2009; Larouche et al., 2015). RV-coefficient analysis is a robust method to test whether *a priori* defined modules fit observed covariance matrices better than a distribution of randomly generated modules of equivalent size and number (Goswami and Polly, 2010a). The RV coefficient is analogous to a correlation coefficient, but instead of measuring the association between two individual variables, it measures the association between two sets of variables. It ranges from zero to one, with zero representing a case

of perfect modularity, in which there is no covariance between the sets. Thus a lower value in *a priori* defined modules than in randomly generated modules indicate that none of the randomly generated modules are better than the *a priori* defined modules. The validation of this modular configuration was done using the function compare modular partitions in geomorph V.20 (Adams et al., 2014) for R.

Allometric Variation

We tested the relationship between shape and size across species using a multivariate regression of shape variables (RWs) onto the centroid size (CS) (Bookstein, 1991; Monteiro, 1999) using TpsRegr, version 1.41 (Rohlf, 2014).

Ecological and Behavioral Diversity

We collected ecological and behavioral data from published literature for each species, focusing on four major characters. First, we recorded wave exposure, binning this variable into: (1) zero to low, (2) moderate, and (3) high wave exposure. Second, we characterized position in the water column in relation to the substratum as: (1) on the substratum (e.g., territorials/close to the substratum), (2) midwater/close to the surface, or (3) both. Finally we characterized behavior as: (1) perennial territorials, solitary, or couples, (2) schooling non-perennial territorials, or (3) solitary, pairs, or small groups non-perennial territorials (Supporting Information 1).

Phylogenetic Comparative Methods

We examined the evolution of morphological and ecological traits using the time calibrated phylogeny of Frédérick et al. (2013). We hypothesized that evolution should favor integration within the swimming system. So, we used a phylogenetic generalized least square method (PGLS) that incorporated Brownian motion (BM) to test for a correlation between the three functional modules.

To test for a correlation between ecology and morphology, we used PGLS methods that incorporated two models of trait evolution, including BM, in which disparity increases as a function of time (Harmon et al., 2003), and the Ornstein-Uhlenbeck (OU) model that incorporates one or more optimum trait value θ_n and strength of selection α (Beaulieu et al., 2012). In other words, BM model is a completely stochastic model, while the OU-model incorporates both stochastic and stabilizing components (Butler and King, 2004). To identify the pattern of morphological diversification, we compared the fit of a BM-model and OU-model for each one of the three ecological variables: (a) wave exposure; (b) position in the water column in relation to the substratum; and (c) behavior. If these ecological variables shaped the evolution of damselfish morphology, we expected that the OU-model would show better fit than the BM model. To evaluate the best model for our data, we compared the fit of both models using Akaike's information criterion (AIC), which considers the trade-off between the fit and the complexity of the model (Akaike, 1973).

For PGLS analysis, we used packages in the R environment version 3.0.3 (The R Foundation for Statistical Computing 2014): NLME (Pinheiro et al., 2014), GEIGER (Harmon et al., 2008), APE (Paradis et al., 2004), and CAPER (Orme et al., 2011).

RESULTS

Main Axes of Morphological Variation

For each module, we considered the PCs that explained at least 70% of the shape variation (Cangelosi and Goriely, 2007). For the cephalic region, four PCs accounted for 71% of shape variation (PC1 = 30%, PC2 = 22%, PC3 = 11%, PC4 = 8%). In

the trunk, three PCs accounted for 75% of the shape variation (PC1 = 48%, PC2 = 17%, PC3 = 10%). The caudal peduncle was the least complex module with two PCs accounting for 81% of shape variation (PC1 = 68%, PC2 = 13%). For each module, the first axis of shape variation (PC1) was related to an elongation along the rostro-caudal axis of the body. For analyses of the whole body, the first four PCs accounted for 71% of the total variation (PC1 = 41%; PC2 = 12%; PC3 = 10%; PC4 = 8%; Supporting Information 2).

Within the cephalic region (*module 1*) PC1 shows that species with horizontally lengthened cephalic profiles, typically have a snout at the same height or higher than the eyes, big eyes, an inferior region of the cephalic profile that is elongated downwards and backwards, a supraoccipital crest (SLMs 17 and 18) that is angled backwards and a more angular opercular margin (e.g., *Azurina* and *Lepidozygus*; Fig. 2B, PC1-). Conversely, species with horizontally short and vertically long cephalic profiles present the opposite character states (e.g., *Dascyllus*; Fig. 2A, PC1+). The second axis of variation shows that in species with big eyes close to the snout, the frontal area of the cephalic region is biggest and an elongated supraoccipital crest (SLMs 17 and 18; e.g., *Azurina* and some *Chromis* species; Fig. 2A,B, PC2+). In contrast, species with smaller eyes located below the snout present the opposite character states (e.g., *Amphiprion*; Fig. 2A, PC2-). The third axis of variation shows that in species with big eyes close to the snout, the frontal area is higher, producing a more rounded cephalic profile (e.g., some *Pomacentrus*, *Chrysiptera Chromis*, and *Neopomacentrus* species; Fig. 2C, PC3-), while species with smaller eyes located below the snout present the opposite character (e.g., some *Abudefduf* and *Microspathodon* species; Figs. 2C and 3D, PC3+). Finally, PC4 segregates species with big eyes and almost round cephalic profiles (e.g., some *Chromis*, *Dascyllus*, and *Pomachromis* species; Figs. 2C and 3D, PC4+) from medium sized eyes with and deep-narrow cephalic profiles (e.g., some *Abudefduf* and *Lepidozygus* species; Figs. 2C and 3D, PC4-).

The first axis of variation of the trunk (*module 2*) is related to an elongated profile of the trunk region resulting from a dorsal/ventral compression of the trunk, with greater compression in the center and anterior edges and less in the posterior. This morphology is associated with an elongated base of the pectoral fin, which is positioned anterior to the pelvic fin and the origin of the dorsal fin (e.g., some *Lepidozygus* and *Azurina* species; Fig. 3B, PC1+). The opposite morphology, shorter profile of the trunk region, is associated with the PC1- axis (e.g., *Dascyllus*; Fig. 3A, PC1-). The second axis of variation shows that in species with pectoral fins positioned anterior to the pelvic fin and origin of the dorsal fin, the dorsal and anal

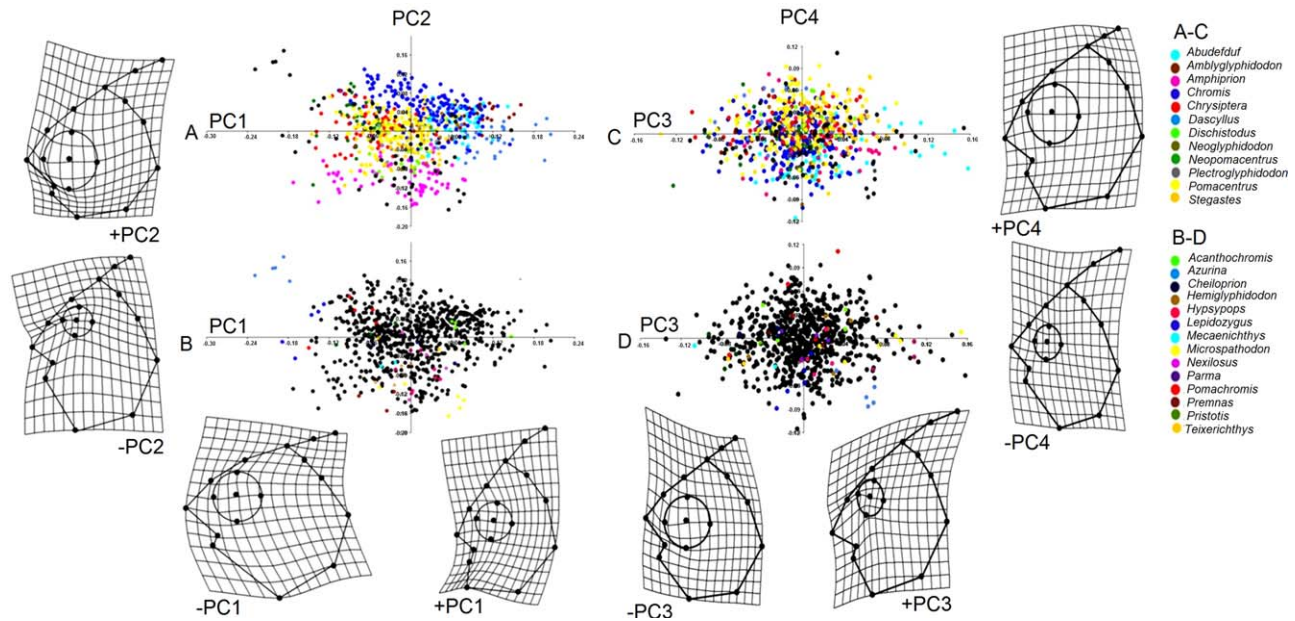


Fig. 2. Principal components analysis of the cephalic region. To highlight genera with few species the same graph is presented twice. Graph A includes all genera with more than five species while graph B only includes genera with less than five species. PC, principal component. Thin plate spline deformation grids represent the deformation of the most extreme specimen in each direction of the PC axes versus the grand mean.

fins are typically short resulting in a longer ventral distance between the pelvic and anal fin (e.g., some *Abudefduf* and *Azurina* species; Fig. 3A,B, PC2–). The opposite morphology, more elongated dorsal and anal fin, is associated with the PC2+ axis (e.g., some *Amphiprion*, *Chromis*, and *Premnas* species; Fig. 3A,B, PC2+). Finally, PC3 show that in species with pectoral fin positioned approximately at the same high of the pelvic fin, the anal fin is typically long and the dorsal fin short (e.g., some *Lepidozygus* species; Fig. 3D, PC3+). The opposite morphology, pectoral fins positioned anterior of the pelvic fin, more elongated dorsal fin and shorter anal fin, is associated with the PC3– axis (e.g., some *Stegastes*, *Chrysiptera*, and *Neophomacentrus* species; Fig. 3C, PC3–).

The main axis of variation of the caudal peduncle (*module 3*) is elongation and degree of curvature. The first axis of variation shows that long caudal peduncles are more curved (e.g., *Azurina*; Fig. 4B, PC1–) than the short ones (e.g., some *Abudefduf*; Fig. 4A, PC1+). The second axis of variation shows that species with an elongated ventral edge present a less curved posterior edge (e.g., some *Chromis* species; Fig. 4A, PC2+), while species with a shorter ventral edge present a more curved posterior edge (e.g., *Amphiprion*; Fig. 4A, PC2+).

For the analyses of whole body shape, the first axis of variation showed that in elongated species where the eye and the snout are at a similar height, the pectoral fin is anterior to the pelvic fin and the dorsal fin origin. There is a general elongation of the cephalic region and caudal peduncle in a verti-

cal axis, in relation to the trunk (e.g., *Azurina* and *Lepidozygus*; Fig. 5B, PC1+). The opposite characters, deep-bodied species, are associated with PC1– axis (e.g., *Dascyllus*; Fig. 5A, PC1–). The PC2 showed a segregation among species with relatively elongated cephalic shape, less curved caudal peduncle, shorter anal and dorsal fins, and pectoral fins in a less horizontal angle of insertion (e.g., some *Abudefduf*, *Chromis*, and *Azurina* species; Fig. 5A,B, PC2–). The PC2+ present the contrary characters, elongated anal and dorsal fin (e.g., *Premnas* and *Pristotis*; Fig. 5B, PC2+).

Inter-Specific Allometry

Results showed CS was a significant predictor of shape variation ($P < 0.001$), but only for a low percentage of shape variation. The trunk shape was most strongly correlated to size ($r^2 = 0.3$; $df = 38–27740$; $F = 350$), such that elongated trunks tend to be smaller than deep-trunks. A smaller proportion of shape variation was explained by size variation in the caudal peduncle ($r^2 = -0.08$; $df = 12–8760$; $F = 61$) and the cephalic region ($r^2 = 0.02$; $df = 30–21900$; $F = 12$). Larger caudal peduncles tend to be more curved than the smaller ones, and larger cephalic regions are generally more vertically elongated than smaller ones (Fig. 6).

Test of Modular Configuration Defined A Priori

The RV-coefficient value obtained for *priori* defined modules ($RV = 0.57$; $P = 0.01$; Supporting

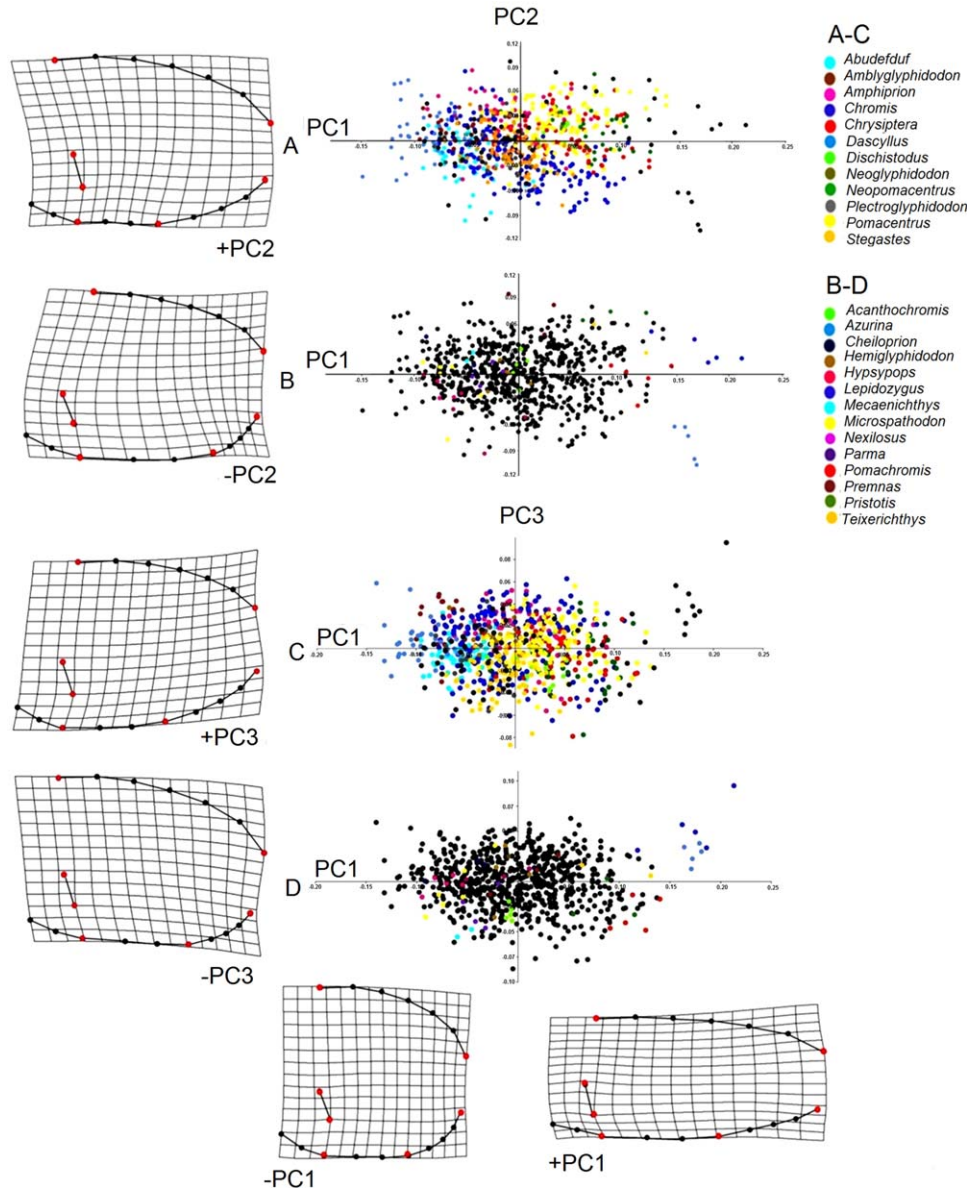


Fig. 3. Principal components analysis of the trunk. To highlight genera with few species the same graph is presented twice. Graph A includes all genera with more than five species while graph B only includes genera with less than five species. PC, principal component. Thin plate spline deformation grids represent the deformation of the most extreme specimen in each direction of the PC axes versus the grand mean. Red dots in the grids represent fin insertions.

Information 3) is lower than any randomly generated modules ($RV = 0.69-1$) indicating that our *a priori* defined modules are a better option of modules segregation than any randomly generated possibility.

Modular Evolution

PGLS analysis revealed limited relationship among modules, supporting a modular evolution of the body parts (Table 1). In total, only 16 of 36 comparisons revealed a significant correlation between axis of variation in the three modules and phylogenetic relationship. Although each module

is significantly related to at least one other module ($P \leq 0.05$), the correlation values were always very low (r^2 value ≤ 0.02 , Table 1).

Ecological Driving Forces

Models of trait evolution showed strong support for the OU-model mainly in the second, third, and fourth axes of variation (PC2, 3, and 4; Table 2) across all modules and all ecological variables. Behavior and position in the water column are related to cephalic region and caudal peduncle in axes representing a high percentage of the total variation (behavior, *module 1*, PC2 = 23% and

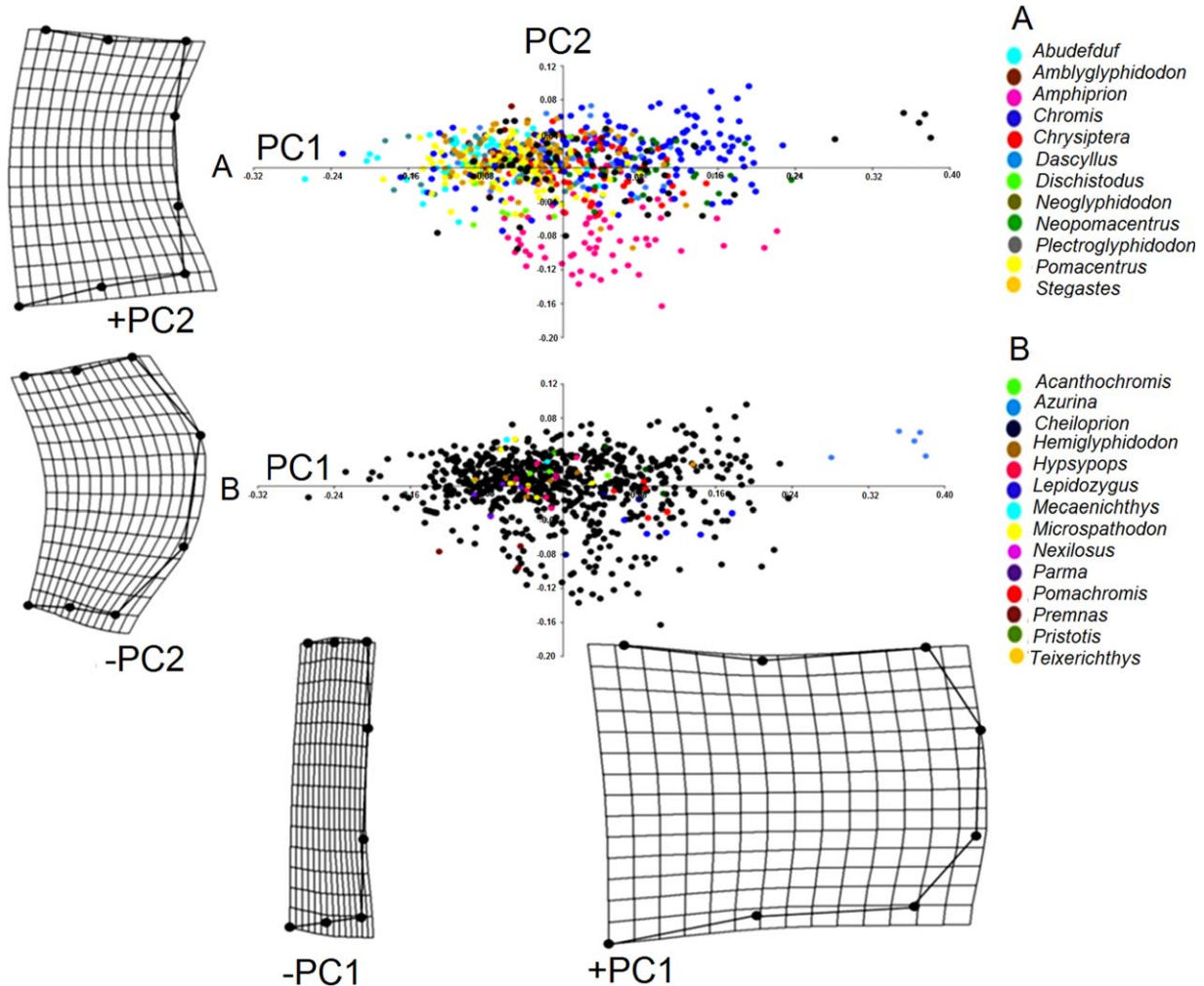


Fig. 4. Principal components analysis of the caudal peduncle. To highlight genera with few species the same graph is presented twice. Graph A includes all genera with more than five species while graph B only includes genera with less than five species. PC, principal component. Thin plate spline deformation grids represent the deformation of the most extreme specimen in each direction of the PC axes versus the grand mean.

module 3, PC2 = 13%; position in the water column, *module 1*, PC3 = 11% and *module 3*, PC2 = 13%). Conversely, wave exposure is related to all modules, in axes representing a lower percentage of the total variation (*module 1*, PC3 = 11% and PC4 = 7%, *module 2*, PC3 = 10% and *module 3*, PC2 = 13%; see the morphological variation related to each axis on Results; Main axes of morphological variation). Thus, our results clearly indicate that the ecological variables analyzed here influenced the shape of major anatomical modules during the evolutionary processes of the Pomacentridae family.

DISCUSSION

Although organisms are commonly viewed as integrated morphological units, they can also be viewed as a collection of semi-autonomous modules with unique positions and functions. Therefore,

understanding the adaptation, evolution, and divergence within groups of organisms requires understanding how the underlying morphological and functional interactions act on these units (modules) and how changes in one unit may impact other units and functions across phylogenies (Zelditch and Carmichael, 1989; Marroig and Cheverud, 2001; Young and Hallgrímsson, 2005; Goswami, 2006a,b; Klingenberg, 2008; Singh et al., 2012).

By examining morphometric and ecological data from 204 species of damselfishes (including 27 of 29 genera) in a comparative phylogenetic framework, this study found evidence for modular evolution in the locomotor system of damselfishes. Specifically, results show that the modules of the damselfish locomotor system are constrained by behavior and habitat preferences. The integration of these modules produces internal constraints on the overall body morphology, resulting in distinctive morphologies. However, the integration among

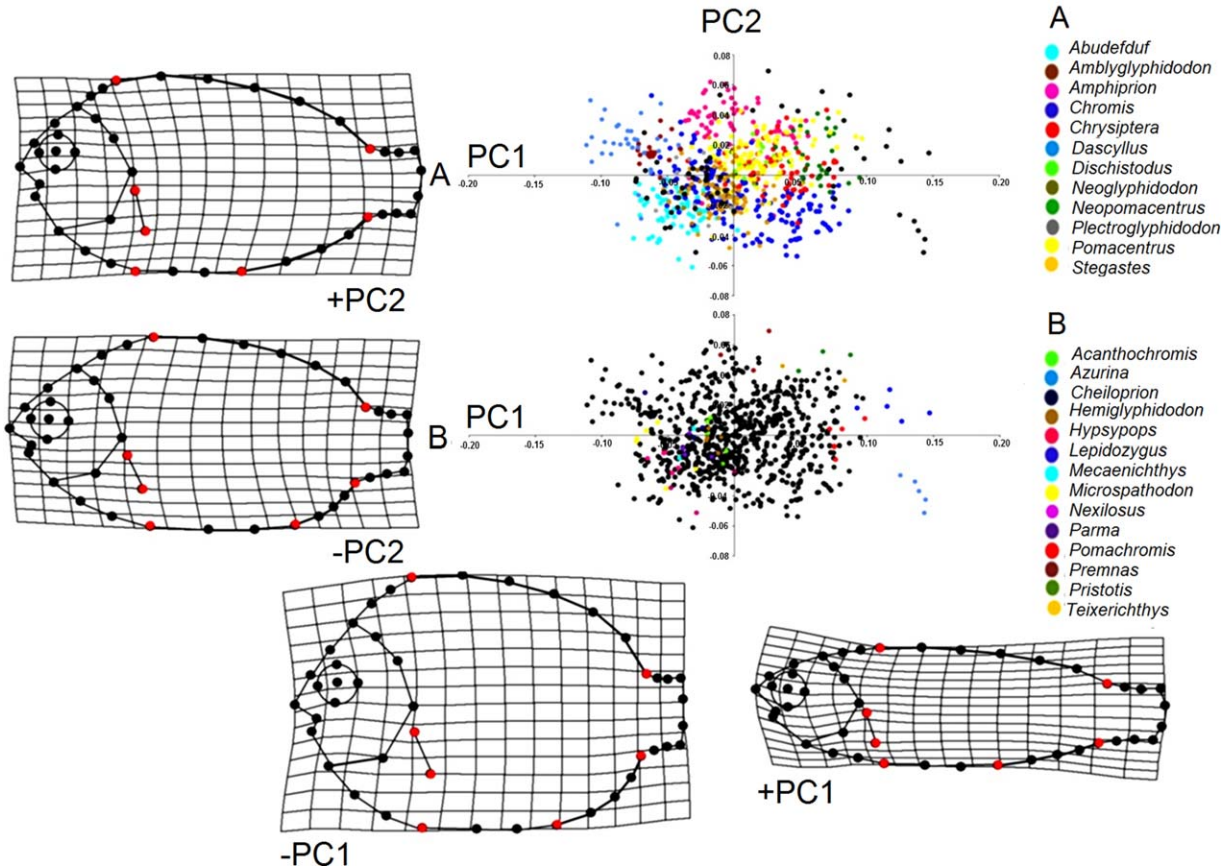


Fig. 5. Principal components analysis of the whole body. To highlight genera with few species the same graph is presented twice. Graph A includes all genera with more than five species while graph B only includes genera with less than five species. PC, principal component. Thin plate spline deformation grids represent the deformation of the most extreme specimen in each direction of the PC axes versus the grand mean. Red dots in the grids represent the fins.

locomotory modules is low (Table 1), potentially providing flexibility in generating morphological diversity that could contribute to species diversity.

Modularity and Ecological Constraints on Locomotor System Shape of Damselfishes

There are different ways to conceptualize morphological modules, including origins during development (Monteiro et al., 2005), integration among LMs with similar patterns of variation (Goswami, 2006a,b), and interactions of traits that perform one or more coordinated functions (Klingenberg, 2008). The latter “functional modality” definition (Mitteroecker and Bookstein, 2007) is more appropriate for characterizing the damselfish locomotor system because the cephalic region, trunk, and caudal peduncle collectively serve a common functional role, are integrated, and relatively independent from other modules.

Each module was described according to its own functions; however they all serve the same purpose, locomotion. For example, the trunk, dorsal,

pelvic and anal fins are associated with stability and thrust (Harris, 1938; Drucker and Lauder, 2001, 2005; Fulton, 2007; Standen, 2008), while the caudal peduncle is associated with acceleration and thrust (Webb, 1982; Gibb et al., 1999), however, both trunk and caudal peduncle function together to increase and keep speeds during locomotion. Thus, even if our results show that anatomically nearby modules are more related among it, these results are related to share functions.

To our knowledge, only few studies have analyzed morphological modularity of fishes considering the whole body. Those analyses resulted in somewhat diverging results. Mabee et al. (2002) considered the anal and dorsal fins as a single module and the pelvic and pectoral fins separately. Larouche et al. (2015) just two modules in the whole body, the cephalic region as one module, and trunk-caudal peduncle, including all fins as the second module. Our results partially agreed with both suggestions. We found a strong relation among the caudal and dorsal region, and a relation among the pectoral and pelvic fins (Fig. 3).

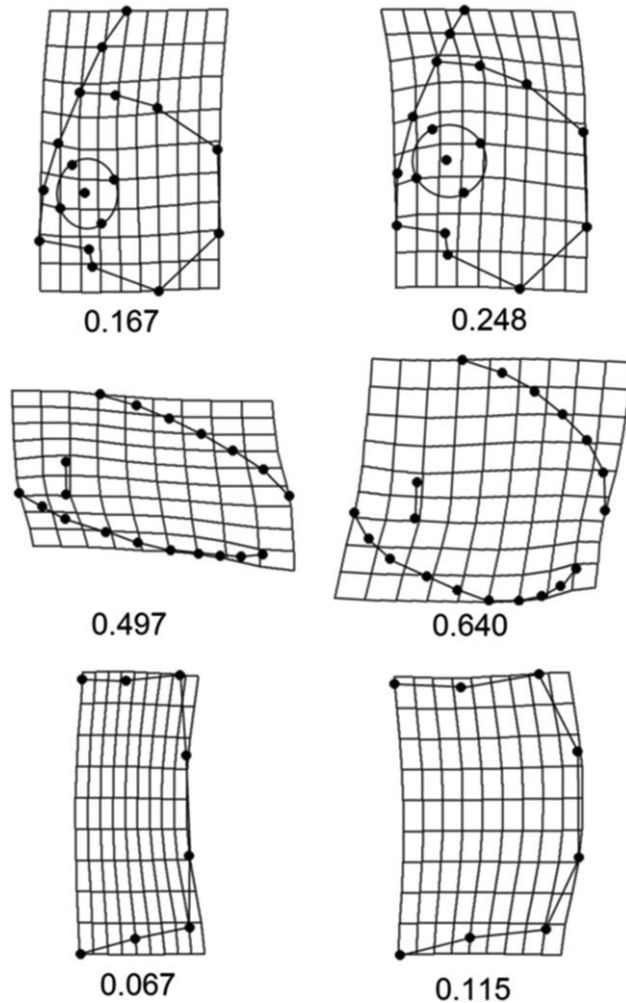


Fig. 6. Morphological variation related to size in the three modules of the locomotor system of damselfishes. Numbers represent the centroid size.

Also when we found a level of integration among the trunk and the caudal peduncle, our result still support the presence of three modules in the whole body.

The largest source of morphological variation observed in the damselfish locomotor system was related to the rostro-caudal elongation of the fish body, a major trend in shape diversity in reef fishes (Ward and Mehta, 2010; Aguilar-Medrano et al., 2011; Aguilar-Medrano, 2013; Claverie and Wainwright, 2014). Although, it is interesting to note that the three studied ecological variables do not show significant relationships with the first axis of variation but instead with the secondary axes. This might indicate that the rostro-caudal elongation of the body presents a strong phylogenetic signal in this fish group, while all secondary characters have been constrained by ecology.

Most elongated damselfish were largely schooling pelagic feeders, inhabiting the midwater column or close to the surface (Supporting Information 1). These species are generally small, and their elongated body tended to be associated with angular cephalic regions, creating a more streamlined profile that decreases drag and increases swimming speeds (Bushnell and Moore, 1991; Lighthill, 1993). This morphology was also associated with big eyes that are level with the snout, a common characteristic of predatory fishes that improves the efficiency of feeding (Griffin et al., 2001; Karplus and Algom, 1981). Lastly, elongated body forms were associated with an elongated caudal peduncle that is curved on the horizontal edge and posterior edge, creating a large area of thrust that promotes rapid acceleration (Webb, 1984; Webb and Weihs, 1986; Lauder, 2000). The combination of these morphological traits results in species that are fast and efficient swimmers, capable of rapid accelerations (e.g., *Azurina* and *Lepidozygus*), characteristics required to successfully exploit midwater habitats as well as those close to the surface.

In contrast, most deep-bodied damselfishes are territorial, benthic feeders, and inhabit environments close to the substratum (Frédérich et al., 2008; Cooper and Westneat, 2009; Aguilar-Medrano et al., 2011), and are larger than

TABLE 1. Results from phylogenetic generalized least squares analyses using a Brownian motion model for testing correlated evolution between morphological modules: Module 1, cephalic region; Module 2, trunk; and Module 3, caudal peduncle

		Cephalic region				Trunk			Caudal peduncle
		PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC1
Cephalic región	PC1								
	PC2	ns							
	PC3	ns	ns						
	PC4	ns	ns	0.01*					
Trunk	PC1	0.02*	0.02*	0.02*	ns				
	PC2	-0.01*	-0.01*	-0.01*	ns	-0.01*			
	PC3	ns	-0.02*	-0.02*	ns	ns	-0.02*		
Caudal peduncle	PC1	-0.01*	-0.01*	-0.01*	ns	-0.01*	-0.01*	ns	
	PC2	ns	Ns	ns	ns	ns	ns	ns	ns

Significant correlations are indicated by an asterisk, while ns indicate no significant results. PCs refer to each principal component.

TABLE 2. Results of phylogenetic generalized least squares analyses testing correlated evolution between morphology and ecology

			Wave exposure		Position in the water column		Behavior		
			AIC	Corr.	AIC	Corr.	AIC	Corr.	
Whole body	PC1	BM	-812.08	0.004*	-806.45	0.000	-809.17	-0.001	
		OU	-687.34	0.003	-686.84	0.003	-690.27	0.009	
	PC2	BM	-831.40	0.000	-846.50	0.005*	-833.32	0.002	
		OU	-936.07	-0.006*	-930.93	0.002	-931.29	-0.001	
	PC3	BM	-831.28	0.002	-836.26	0.003*	-831.96	0.002	
		OU	-1,047.55	-0.001	-1,046.14	0.000	-1,049.23	-0.003	
	PC4	BM	-1,018.83	0.000	-1,019.09	0.001	-1,023.00	-0.005	
		OU	-1,059.78	0.003	-1,057.73	-0.002	-1,079.59	-0.009*	
Module 1 Cephalic región	PC1	BM	-605.10	0.008*	-606.13	0.006*	-601.40	0.001	
		OU	-552.53	0.000	-553.62	0.006	-570.03	-0.029*	
	PC2	BM	-649.38	0.003	-664.71	-0.008*	-651.80	0.009	
		OU	-610.05	-0.001	-610.89	-0.005	-655.71	0.040*	
	PC3	BM	-623.04	-0.012*	-616.40	0.006*	-611.70	-0.002	
		OU	-800.40	-0.007*	-804.24	0.007*	-796.20	-0.001	
	PC4	BM	-642.36	-0.004	-699.05	0.014*	-643.41	-0.006	
		OU	-794.05	-0.007*	-788.80	0.000	-790.81	-0.004	
Module 2 trunk	PC1	BM	-726.34	-0.009*	-712.94	-0.001	-715.48	-0.002	
		OU	-599.56	-0.005	-598.19	0.002	-603.65	-0.014*	
	PC2	BM	-774.47	0.005*	-770.41	-0.001	-772.61	-0.004	
		OU	-833.52	0.006	-829.78	0.002	-831.17	-0.003	
	PC3	BM	-863.82	0.000	-863.90	0.001	-868.08	-0.007	
		OU	-952.95	0.005*	-947.45	-0.001	-950.05	-0.003	
	Module 3 Caudal peduncle	PC1	BM	-497.20	0.006	-496.62	0.004	-497.55	0.003
			OU	-458.71	-0.006	-468.17	0.018*	-458.81	-0.005
PC2		BM	-636.52	0.005	-636.36	0.004	-635.89	-0.001	
		OU	-790.93	0.009*	-793.38	-0.007*	-799.06	0.015*	

Two models were used, BM (Brownian motion) and OU (Ornstein-Uhlenbeck) and compared using Akaike information criterion scores (AIC). Corr.: correlation values. Bold values indicate the best fitted model. Asterisk indicated significant correlation ($P = < 0.05$). Dashed circled values indicated significant correlation on BM as best fitted model and solid circled values indicated significant correlation on OU as best fitted model.

schooling damselfishes. Results showed that deep-bodied species generally have deep cephalic profiles with lower positioned snouts that allow them to graze on algae, or prey on benthic invertebrates while maintaining visual awareness of predators (Bellwood et al., 2014). In addition, these species typically have short caudal peduncles that facilitate strong initial swimming bursts rather than sustained fast swimming required of midwater species. Furthermore, these species have deep-trunks that favor stability at slow swimming speeds, and are likely advantageous for negotiating structurally complex habitats (e.g., *Dascyllus aruanus* living in branching corals; Webb, 1982; Fulton, 2007).

Despite this generality, numerous taxa deviated from this pattern. For example *Amphiprion* presented a rounded cephalic profile, a trait commonly observed in benthic species, but its trunk and caudal peduncle were relatively long and similar to species living in the upper and mid-water column. In contrast, *Hypsypops* exhibited an angular cephalic profile, characteristic of species from the water column, but also had a high trunk and short caudal peduncle, traits generally associated with benthic habitats. Thus, while the evolution of pomacentrids is biased in specific phenotypic directions due to general rules that link the evolution

of modules of the locomotor system (Klingenberg, 2005), these links are flexible. The low association among modules allows morphological diversity to increase by producing unexpected combinations. In turn, this variation may facilitate shifts into novel niche spaces, contributing to lineage diversification in pomacentrids.

Previous studies have demonstrated the relationship between cephalic shape and diet in damselfishes (Emery, 1973; Gluckmann and Vandewalle, 1998; Aguilar-Medrano et al., 2011; Frédérick et al., 2013). While the ecological variables studied in our analysis are highly related to locomotion, they also influence feeding behavior. For example, deep-bodied species with short caudal peduncles were typically associated with slower, more maneuverable swimmers (Bartol et al., 2002; Fulton, 2007; Aguilar-Medrano, 2013). In turn, these characteristics were associated with being territorial and herbivorous (Frédérick et al., 2008; Cooper and Westneat, 2009; Aguilar-Medrano et al., 2011). Similarly, results showed that elongated species with longer caudal peduncles were associated with faster, more efficient swimming (Videler, 1993; Fulton, 2007; Aguilar-Medrano, 2013); these species were typically schooling and mostly zooplanktivorous (Frédérick et al., 2008; Cooper and Westneat,

2009; Aguilar-Medrano et al., 2011). The strong connections observed between locomotion and feeding in damselfishes (see above) is similarly seen in other fishes, such as the Labridae (Collar et al., 2008) suggesting that constraints of swimming and feeding in an aquatic medium can have similar effects across distantly related taxa.

Morphological Evolution of the Damselfish Locomotor System

Our study system highlights the balance between evolvability and morphological constraints. Evolvability is the potential of populations to respond to natural selection and evolve (Wagner and Altenberg, 1996; Klingenberg, 2005; Singh et al., 2012) rising to novel phenotypes, while the morphological constraints imposed by integration of morphology within locomotor modules reduces the potential to vary within a population, conserving aspects across evolutionary time (Klingenberg, 2005; Singh et al., 2012).

Evolutionary analysis of damselfish morphology illuminates a limited range of morphological variation and a tendency to repeat shapes through clades (Cooper and Westneat, 2009; Frédérick et al., 2013). In particular, the integration among the cephalic region, trunk, and caudal peduncle suggest the action of morphological constraints because the integration of modules potentially limits the variation of each module (Martin et al., 2005). As such, evolutionary change is biased in specific phenotypic directions (Klingenberg, 2005), potentially slowing the rate of evolution and/or constraining morphological evolution to a smaller range of possible variation (Goswami and Polly, 2010b). Thus, the integration among modules may explain the limited range of morphological variation in pomacentrids (giving this group their distinctive shape) and the tendency to reiterate patterns throughout the evolutionary history of this group.

While results suggest integration among modules, a high percentage of the morphological variation within damselfishes shows little integration. The freedom to evolve more independently from the main body pattern may result in greater morphological variation and species diversity in some lineages of pomacentrids. For example, a higher level of modularity has probably operated for the evolution of *Amphiprion*. This genus combines rounded cephalic profile with elongated trunk and caudal peduncle, and this atypical combination of shapes may be considered as a novelty in damselfishes. In addition to physiological adaptations, this modular novelty could be related to the colonization of untapped niches such as sea anemones and could explain the high rate of diversification observed in the clade of clownfishes (Litsios et al., 2012). Such “independent” characters are likely critical for evolvability by increasing the potential for morphological variation (Goswami and Polly, 2010b).

As expected, behavior and habitat preferences impact the morphological variation of each module of the fish body during the evolution. Thus, selection pressure and integration of damselfish locomotory modules should result in a relatively low number of morphological possibilities and correspondingly limited morphological diversity. However, evolution has produced lineages of damselfish that do not seem to follow these constraints. Hence the results suggest that the evolutionary success of Pomacentridae may lie in the delicately balanced interaction between integrated parts of the morphology that produce a functional body design and weakly integrated or relatively independent morphological aspects that facilitate adaptation and diversification.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

None of the organisms used in this analysis were collected in the field. Most photographs were obtained from accessioned museum specimens, with additional photographs obtained from online resources such as MorphBank (www.morphbank.net), FishBase (Froese and Pauly 2014), and John E. Randall's Fish Photos (pbs.bishopmuseum.org/images/JER).

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