Iterative Ecological Radiation and Convergence during the Evolutionary History of Damselfishes (Pomacentridae)

Bruno Frédérich,[1,*] Laurie Sorenson,[2] Francesco Santini,[2] Graham J. Slater,[2] and Michael E. Alfaro[2]

1. Laboratoire de Morphologie Fonctionnelle et Evolutive, Institut de Chimie (B6c), Université de Liège, B-4000 Liège, Belgium;
2. Department of Ecology and Evolutionary Biology, University of California, Los Angeles, California 90095

abstract: Coral reef fishes represent one of the most spectacularly diverse assemblages of vertebrates on the planet, but our understanding of their mode of diversification remains limited. Here we test whether the diversity of the damselfishes (Pomacentridae), one of the most species-rich families of reef-associated fishes, was produced by a single or multiple adaptive radiation(s) during their evolutionary history. Tests of the tempo of lineage diversification using a time-calibrated phylogeny including 208 species revealed that crown pomacentrid diversification has not slowed through time as expected under a scenario of a single adaptive radiation resulting from an early burst of diversification. Evolutionary modeling of trophic traits similarly rejected the hypothesis of early among-lineage partitioning of ecologically important phenotypic diversity. Instead, damselfishes are shown to have experienced iterative convergent radiations wherein subclades radiate across similar trophic strategies (i.e., pelagic feeders, benthic feeders, intermediate) and morphologies. Regionalization of coral reefs, competition, and functional constraints may have fueled iterative ecological radiation and convergent evolution of damselfishes. Through the Pomacentridae, we illustrate that radiations may be strongly structured by the nature of the constraints on diversification.

Keywords: coral reef fishes, disparity, geometric morphometrics, morphospace, Ornstein-Uhlenbeck, repeated adaptive radiations.

Introduction

Understanding the factors responsible for contemporary patterns of morphological diversity and species richness is a fundamental challenge of evolutionary biology. One mechanism that is commonly invoked to explain the presence of particularly successful and ecologically diverse clades is that of adaptive radiation. As originally envisioned by Simpson (1944), adaptive radiation involves the early, rapid diversification of multiple lineages from a common ancestor into new, diverging adaptive zones. Simpson

imagined that adaptive radiations played an important role in the origin of higher taxa; over time, however, the term adaptive radiation has come to refer to any clade experiencing rapid lineage diversification (or cladogenesis) and strong phenotypic differentiation among subclades as a consequence of ecological opportunity generated by colonization of new areas, extinction of competitors, or the development of key innovations (Schluter 2000; Losos 2010). Textbook examples of this kind of adaptive radiation include Darwin's finches on the Galápagos Islands and cichlids of the east African Great Lakes.

Although many striking radiations have been labeled as adaptive radiations, several recent empirical studies have raised questions about the generality of the predicted outcomes (i.e., rapid phenotypic evolution and lineage diversification). In a study of cetacean diversification, Slater et al. (2010) found no evidence for an early burst of lineage diversification in the cetacean phylogeny. However, they did find evidence for a slowdown in rates of body size evolution, consistent with niche filling expected under adaptive radiation, leading them to suggest that high rates of turnover might erase the signal of adaptive radiation from molecular phylogenies while preserving signal in phenotypic data sets. Dornburg et al. (2011) found a similar pattern in extant triggerfishes, where an innovative mode of locomotion drove an early radiation of shape and function but not an early radiation of species. Harmon et al. (2010) fit models of trait evolution to 49 comparative data sets derived from a variety of animal clades yet found little support overall for a model with an early burst of phenotypic evolution. As a result, they suggested that this classical model of adaptive radiation may be rare in comparative data (Harmon et al. 2010).

Despite a general dominance of the "early burst" paradigm for adaptive radiation in current evolutionary research, a variety of alternative definitions have been proposed that do not explicitly require such a pattern (e.g., Givinish and Systema 1997; Losos 2009; Olson and Ar-

royo-Santos 2009). Clades may adaptively radiate through alternative scenarios such as repeated, convergent radiations. This kind of adaptive radiation may occur in systems dominated by constraints, such as when evolution within clades is driven by repeated adaptation to similar environments offering the same set of available niches. Developmental processes, pleiotropic effects, morphological integration or competition can be seen as sources of constraint leading to convergences and repeated radiations (Losos 2011). Under this repeated radiation scenario, adaptive radiations are replicated, producing multiple, similar sets of descendant species. Importantly, hypotheses of repeated adaptive radiation predict no early burst of lineage diversification, and subclades tend to resemble one another in morpho-functional disparity, the exact opposite pattern of that predicted under the early burst paradigm. The Caribbean *Anolis* lizards, although often cited as a classical adaptive radiation, are a prime example of repeated radiations (Losos et al. 1998). There is no evidence for an early burst of lineage or trait diversification in these taxa, but both appear to be strongly diversity dependent within islands (Mahler et al. 2010; Rabosky and Glor 2010). Generally speaking, the dynamics of unconstrained systems (i.e., those with lots of open niches driving early burst of diversification with little convergence among subclades) versus systems dominated by constraints (i.e., those with a well-circumscribed set of available niches driving convergences) have not really been explored and the description of signatures allowing their distinction will be helpful for comparative biologists studying the dynamics of clade diversification.

Coral reefs are known for their high level of biodiversity, especially in fishes. Recent studies have showed that coral reefs act as drivers of cladogenesis (Alfaro et al. 2007; Cowman and Bellwood 2011), and as promoters of morphological diversity and ecological novelty (Price et al. 2011). Coral reef fish families have been used as case studies for testing the influence of various factors on the tempo and the mode of diversification during their evolutionary history (e.g., Alfaro et al. 2009*a*; Kazancioglu et al. 2009; Dornburg et al. 2011), but works dedicated to the global exploration of their radiation remain scarce.

Damselfishes (Pomacentridae) comprise 386 species (Froese and Pauly 2012), living mainly in coral reef environments and divided in five subfamilies: Stegastinae, Lepidozyginae (monospecific), Chrominae, Abudefdufinae, and Pomacentrinae (Cooper et al. 2009). In terms of species number, this family represents the third-largest fish group in coral ecosystems after Gobiidae (>1,500 species) and Labridae (>600 species; Wainwright and Bellwood 2002). Damselfishes are locally abundant and appear to be one of the most successful radiations of coral reef fishes. The fossil record shows that they have been present within coral reef ecosystems for at least 50 million years (Bellwood 1996; Bellwood and Sorbini 1996; Carnevale and Landini 2000). Recently, Cooper and Westneat (2009) suggested that damselfishes experienced rapid and repeated diversification into three major trophic guilds: planktivory, herbivory, and omnivory. However, a large sampling of damselfish species, allowing the representation of all trophic groups within each major subclade, is needed to more fully evaluate this idea. Damselfish oral jaw morphology is tightly linked to trophic ecology (Frédérich et al. 2008; Frédérich and Vandewalle 2011), and thus a morpho-functional analysis of trophic characters offers an excellent system for testing whether pomacentrids have experienced iterative adaptive radiations. If damselfish diversity reflects a long history of repeated convergent evolution into a small number of trophic guilds, we expect to see similar ecological and morphological disparity as well as the same patterns of phenotypic diversification among major subclades. This hypothesis has yet to be tested in the context of a large, time-calibrated phylogeny using recently developed comparative methods specifically designed to test for convergence (Adams and Collyer 2009; Adams 2010).

Here we use a suite of phylogenetic comparative and morphometric methods to test whether damselfish diversity can be linked to a single or multiple adaptive radiation(s). We construct a multigene timescale for damselfish evolution incorporating 55% of the extant damselfish species and use it to quantify patterns of lineage diversification and of evolution in phenotypic characters related to their ecology (i.e., body size and oral jaws). Counter to simple predictions of ecological adaptive radiation (Schluter 2000), we find strong evidence for repeated patterns of morphological diversification and a high degree of convergence in the history of damselfish subclades. Additionally we find strong support for constrained evolution where different phenotypic optima are shaped by trophic strategies.

Material and Methods

Taxon Sampling

Our molecular data set includes 208 species (55% of the described damselfish species) representing 28 of the 29 currently described genera (only the monotypic genus *Nexilosus* is missing).

For the molecular analyses, we downloaded sequences from Genbank for three nuclear gene fragments (rag1, rag2, and bmp4) and five mitochondrial gene fragments (12s, 16s, nd3, cox1, and cytb) for 165 pomacentrid species, as well as eight outgroups (table S1, available online). Most of these data derive from Tang (2001), Quenouille et al. (2004) and Cooper et al. (2009). Tissues for 43 ad-

ditional species were secured through fieldwork in Madagascar, purchases from pet trade wholesalers, or loans from the Los Angeles County and University of Kansas Natural History Museums (table S1). New sequence data spanning the eight loci were generated for these species using methods outlined in appendix S1, available online. For the morphological analyses, a combination of specimens collected in the field (Corsica, French Polynesia, Hawaii, Japan, Madagascar, Papua New Guinea, Taiwan) and accessioned museum specimens were studied (table S1). Methods for field collections were described in Frédérich and Vandewalle (2011) and followed approved animal care protocols.

Time Tree Inference

Phylogenetic reconstructions performed using maximum likelihood analyses with RAxML (Stamatakis 2006) and Bayesian analyses using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) are detailed in appendix S1.

   We used BEAST 1.6.2 (Drummond and Rambaut 2007) to infer phylogeny and divergence times. Five fossils were used to time-calibrate the molecular phylogeny. We used *Morone* sp., the oldest fossil assigned to moronids from the Late Campanian (Late Cretaceous, 74–73 Ma; Nolf and Dockery 1990) to date the split between *Dicentrarchus labrax* and all other groups in our data set and the otoliths assigned to the genus "*Epigonidarum*" *weinbergi* from the Coniacian (Late Cretaceous, 89–84 Ma) to establish an upper boundary (offset = 74, mean = 8.0). *Palaeopomacentrus orphae* and *Lorenzichthys olihan* from the Ypresian (Eocene, 50 Ma; Bellwood 1999) were used to determine the minimum age of the crown pomacentrids (offset = 50, mean = 12.0). *Chromis savornini* from the late Miocene (6.5 Ma; Arambourg 1927) was used to date the origin of the genus *Chromis*. Due to the likely young age of this fossil we assigned a very loose upper bound to this calibration point (50 Ma, age of the oldest crown pomacentrid). Full details of the phylogenetic analyses are provided in appendix S1.

Lineage Diversification

If a single ecological adaptive radiation is the primary factor shaping damselfish diversity, we would predict that species diversification rates would be highest early in the history of the clade and then slow through time as available niches filled (Schluter 2000; Rabosky et al. 2007). We tested this prediction by computing the gamma statistic and assessing significance using the Monte Carlo constant rates (MCCR) test, which accounts for incomplete taxon sampling (Pybus and Harvey 2000). We also assessed the fit of four explicit models of clade accumulation to the

branching times in our phylogeny. We compared two constant rate models (Yule and birth-death) to two alternative models that predict slowdowns in the tempo of diversification (density-dependent exponential [DDX] and density-dependent logistic [DDL]) following Rabosky and Lovette (2008). We used Akaike's Information Criterion (AIC) scores and weights (Burnham and Anderson 2002) to compare the fit of the models. The MCCR test and diversification model fitting were done using the LASER package (Rabosky 2006) for R (R Development Core Team 2011). We also applied MEDUSA (Alfaro et al. 2009b) to identify diversification rate shifts across a backbone phylogeny of damselfish lineages combined with full taxonomic richness data for incompletely sampled clades (i.e., 386 species, according to Froese and Pauly 2012). The AIC cutoff was automatically calibrated based on tree size.

Ecological and Morphological Diversity

We assigned the 208 species to one of three commonly recognized trophic groups (Allen 1991; Cooper and Westneat 2009; Frédérich et al. 2009) based on literature review: (1) pelagic feeders that suck planktonic copepods, (2) benthic feeders that graze filamentous algae or bite coral polyps, and (3) an intermediate group, which feeds on planktonic prey, small benthic invertebrates, and algae in variable proportions. Some benthic feeding damselfishes are also farmers that defend territories and manage dense stands of filamentous algae as their own algal farm (Lobel 1980; Hata and Kato 2002; Ceccarelli 2007). Because our second trophic group contains species that do and do not farm, we have considered this farming behavior as an independent ecological category from trophic groups. A thorough review of the literature, supplemented by unpublished data kindly provided by H. Hata and D. M. Ceccarelli, allowed us to identify 35 farming species, although it is likely that the true number of farming damselfish species is higher. Ecological data are summarized in table S1.

   We collected the maximum standard length (SL, length from the tip of the snout to the posterior edge of the caudal peduncle) from FishBase (Froese and Pauly 2012) and Allen (1991) for all 208 damselfish species. The oral jaws (premaxillary bone and mandible) of 600 adult specimens comprising 129 species of damselfishes (table S1) were dissected and then cleared and stained with alizarin red S (Taylor and Van Dyke 1985). Sample sizes within species ranged between 1 and 22 individuals (median = 3 individuals; table S1). The left mandible and the left premaxilla were photographed using 8–10 megapixel digital cameras installed on binocular microscopes. We used landmark-based geometric morphometric methods (Bookstein 1991; Rohlf and Marcus 1993; Adams et al.

2004) to quantify the shape of the mandible and the premaxilla. Twelve and six homologous landmarks were recorded from the left lateral view of the mandible and the premaxilla, respectively, using TpsDig (Rohlf 2004; fig. 1). All landmarks are described in Frédérich et al. (2008) and the various steps taken to reduce measurement errors are detailed in Frédérich and Sheets (2010). For each skeletal unit, we optimally aligned the specimens of each species using a first generalized Procrustes superimposition (Rohlf and Slice 1990) to generate a mean shape for every species. We then performed a relative warps analysis (Rohlf 1993) on the consensus landmark configuration for each species to generate a morphospace illustrating the major axis of shape variation (relative warps or "warps" hereafter). We used species scores on individual warps for calculating levels of disparity, examining patterns of disparity, and calculating phenotypic trajectories. Five species in the morphological data set were not sampled in our phylogeny (table S1), so we recalculated warps using a superimposition based upon species included in the phylogeny and used those for all phylogenetic comparative analyses.

Measuring the Tempo of Morphological Evolution

Under the hypothesis of a single adaptive radiation, rates of phenotypic evolution are expected to slow through time as niches become saturated. As a result, we would expect morphological variation to be partitioned within rather than among major subclades; that is, clades should exhibit strong signals of ecological and morphological differentiation as a result of divergence early in their evolutionary history (Schluter 2000; Harmon et al. 2003). Conversely, under the hypothesis of iterative radiations, rates of trait evolution should be faster toward the present as lineages evolve to take advantage of novel ecological opportunities. Under this hypothesis, we expect clades to exhibit overlapping patterns of ecological and morphological diversity, although processes that allow taxa in different ecological niches to possess different distributions of traits may provide a better fit than time-dependent processes. We used several comparative methods to test these hypotheses. First we compared the fit of a Brownian motion (BM) model of trait evolution to the phenotypic data with that of an ACDC model (Blomberg et al. 2003) using the fitContinuous function in the GEIGER package (Harmon et al. 2008) for R (R Development Core Team 2011). These models differ in their parameterizations of the rate of morphological evolution. BM is a time-homogeneous process in which morphological disparity increases uniformly as a function of time. Under ACDC, the rate of morphological change is allowed to exponentially increase or decrease over time. If damselfishes underwent a single adaptive radiation (Simpson 1944; Schluter 2000), we expected an
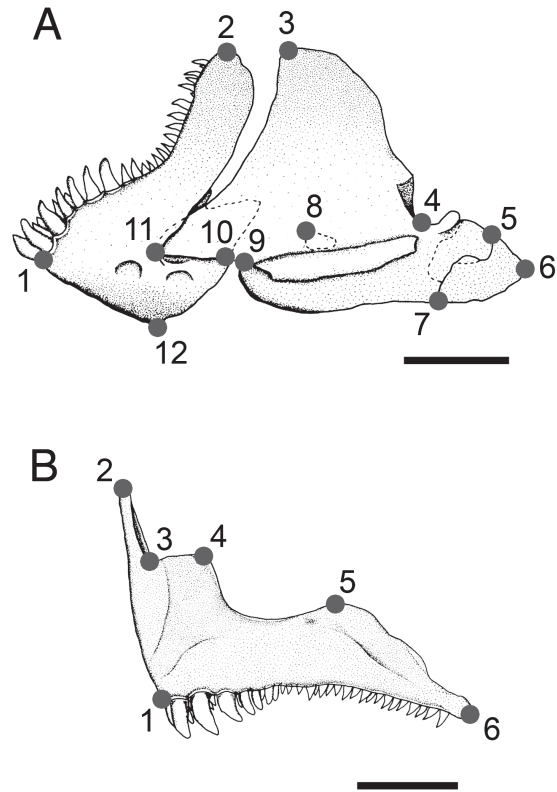

Figure 1: Mandible (*A*) and premaxilla (*B*) of *Dascyllus aruanus*, illustrating the landmarks used for the geometric morphometric analyses. All landmarks are described by Frédérich et al. (2008). Scale bars = 1 mm.

ACDC model with decreasing rate of morphological diversification to fit better than BM (Harmon et al. 2010).

The Ornstein-Uhlenbeck (OU) process is another variation on BM that incorporates one or more optima, θ_n, as well as a so-called rubber-band parameter α that determines the strength with which the trait is drawn back to its optimal value as it evolves away from it. To test whether trophic strategies or farming behavior have significantly shaped patterns of morphological evolution within damselfishes, we also compared the fit of models with a single optimal trait for all damselfishes (OU) to models with separate optima for each trophic strategy (pelagic feeders, benthic feeders, and intermediate—OU_T) or with a separate optimum for farming behavior (farming vs. nonfarming—OU_F) using the OUwie package (Beaulieu et al. 2012) in R (R Development Core Team 2011). If diet or farming behavior constrains fish body size and oral jaw shapes, we would expect OU_T or OU_F to best

fit our data. To account for uncertainty in the evolutionary history of trophic and farming behaviors, we fit the OU_T and OU_F models to 500 stochastic character reconstructions generated using SIMMAP v1.5 (Bollback 2006).

We further tested our prediction of iterative radiations using disparity-through-time analysis (Harmon et al. 2003). This approach computes the average subclade disparity for one or more traits at each node in the phylogeny and plots these as a function of node age. At the root of the tree, the average subclade disparity is simply the morphological disparity of the entire clade and is therefore high. At subsequent nodes, disparity is averaged over the total number of subclades in existence at that time. Under the early burst hypothesis, average subclade disparity is expected to decline rapidly in the early history of the clade as evolutionary rates slow and phenotypic variation becomes partitioned among clades. Conversely, for iterative radiations average subclade disparity should remain high or even increase through time as rates accelerate and variation remains distributed among clades. We used the morphological disparity index (MDI) of Harmon et al. (2003) to quantify the difference between average subclade disparity through time for our observed data set and that expected under a null BM model (Harmon et al. 2003). Negative MDI values indicate lower than expected subclade disparity under BM while positive MDI values indicate higher than expected subclade disparity. MDI statistics were computed for body size and jaw warps scores over the first 80% of the time-tree using GEIGER (Harmon et al. 2008). We omitted the most recent 20% of the phylogeny in our analysis to avoid spurious MDI estimates due to incomplete sampling of tip species (Harmon et al. 2003).

Disparity and Diversification in Morphospace

After a visual exploration of morphospaces defined by relative warps, we calculated the levels of shape disparity exhibited by the four major subclades (i.e., "Stegastinae + the monospecific Lepidozyginae," Chrominae, Abudefdufinae, Pomacentrinae) were calculated and compared. We used the methodology of Zelditch et al. (2003) as implemented in DisparityBox (Sheets 2004) to measure the level of morphological disparity. We then used SpaceAngle (Sheets 2001) to test whether the damselfish subfamilies diversified along the same major axes of shape variation. Detailed information about this test is provided in Zelditch et al. (2004) and Frédérich and Vandewalle (2011).

Comparing the Trajectories of Morphological Evolution

We tested the repeatability of morphological evolution by calculating the trajectories of morphological evolution and

their comparisons among major subclades using the approach of Adams and Collyer (2009). Briefly, this method uses a residual randomization procedure to quantify and compare the size, orientation, and shape of trajectories of phenotypic evolution among taxa in the morphospace. The trajectories of morphological evolution can be defined by two or more evolutionary steps, and only trajectories described by the same number of steps can be compared in the same analysis (Adams and Collyer 2009). In damselfishes, the three trophic ecotypes are the steps; however, because Chrominae does not possess benthic feeders, it cannot be directly compared with the three other main subclades. Additionally, we do not have specimens of Stegastinae belonging to the intermediate ecotype in our morphological data set (table S1). Consequently, we cannot directly compare the trajectories among each major subclade. Thus, in these analyses, we grouped the subclade Stegastinae + Lepidozyginae with Chrominae, and the trajectories of morphological evolution were compared among the Stegastinae + Lepidozigynae + Chrominae, the Abudefdufinae, and the Pomacentrinae. All analytical steps were performed in R (R Development Core Team 2011) following Adams and Collyer (2009).

Results

Damselfish Phylogenetics and Divergence Time Estimation

Our maximum likelihood tree topology is largely concordant with previous analyses (Cooper et al. 2009; fig. S1, available online) and shows *Stegastes*, *Plectroglyphidodon*, *Parma*, *Abudefduf*, *Chromis*, and *Chrysiptera* to be polyphyletic. We recovered three *Chrysiptera* lineages: "*Chrysiptera* I + *Pomachromis richardsoni*," "*Chrysiptera* II + *Cheiloprion labiatus*," and "*Chrysiptera* III + *Amblypomacentrus clarus*." The time-calibrated phylogeny indicates that the origin of the damselfishes occurred in the early Eocene and diversification continued through to the Pleistocene (fig. 2). The chronogram suggests that the stem lineages of the five major subclades (i.e., subfamilies: Lepidozyginae, Stegastinae, Abudefdufinae, Chrominae, and Pomacentrinae) began to diversify between 50 and 25 Ma.

Pattern of Damselfish Species Diversification

Visual inspection of the time-calibrated tree (fig. 2) does not suggest a pattern of high diversification rate in the early history of damselfishes. The gamma statistic for the pomacentrid tree is negative ($\gamma = -4.04$), though not significantly more extreme than would be expected given the degree of sampling in the tree (MCCR test: $P = .79$). The comparison of the fit of the diversification models
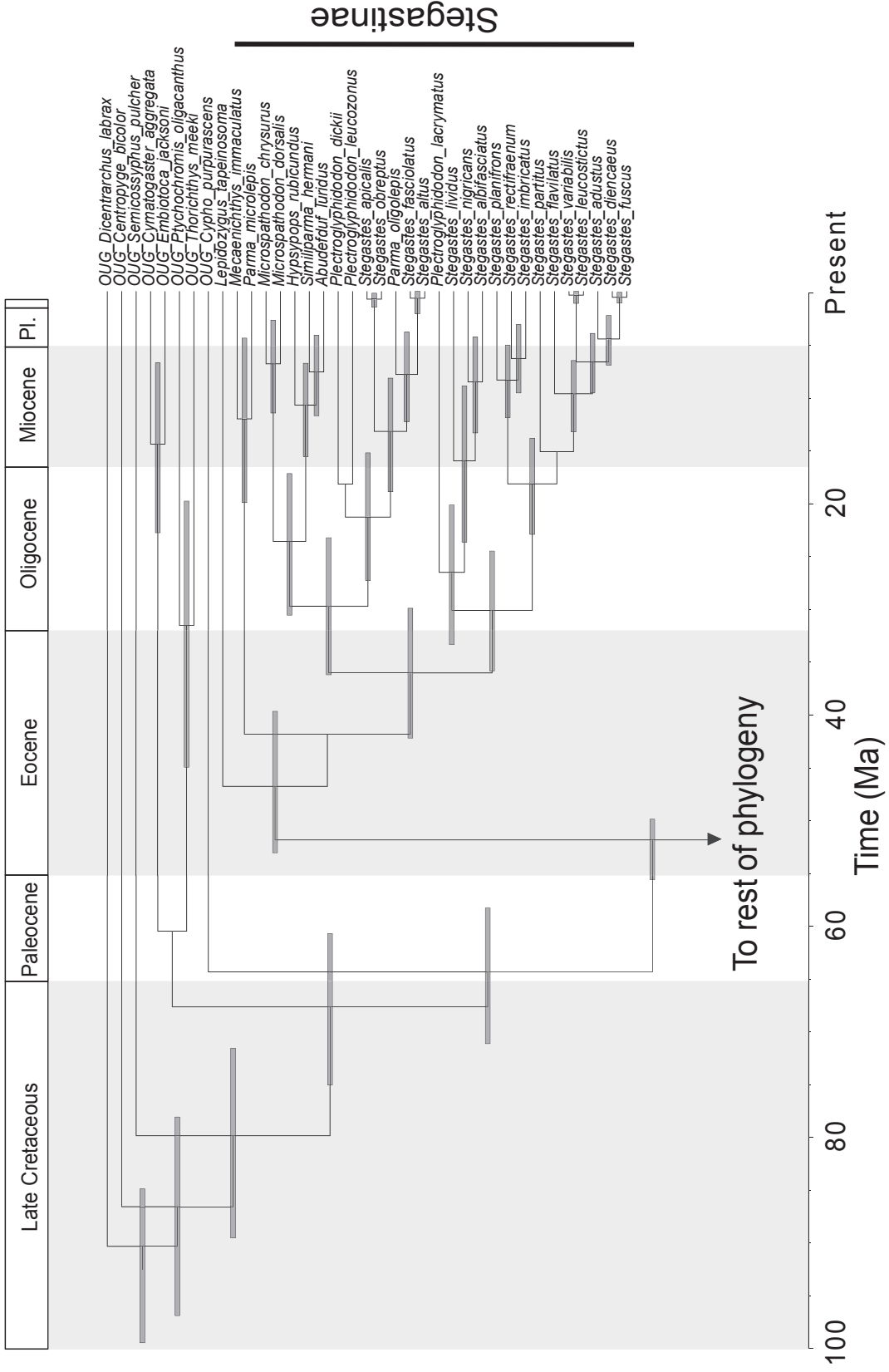
Figure 2: Consensus chronogram of damselfish divergence times. Bars around nodes represent the 95% highest posterior density (HPD). Nodes with posterior probabilities less than 0.5 (see fig. S1, available online) do not contain a 95% HPD on the age estimate. The white star highlights the unique rate shift in cladogenesis identified by MEDUSA.
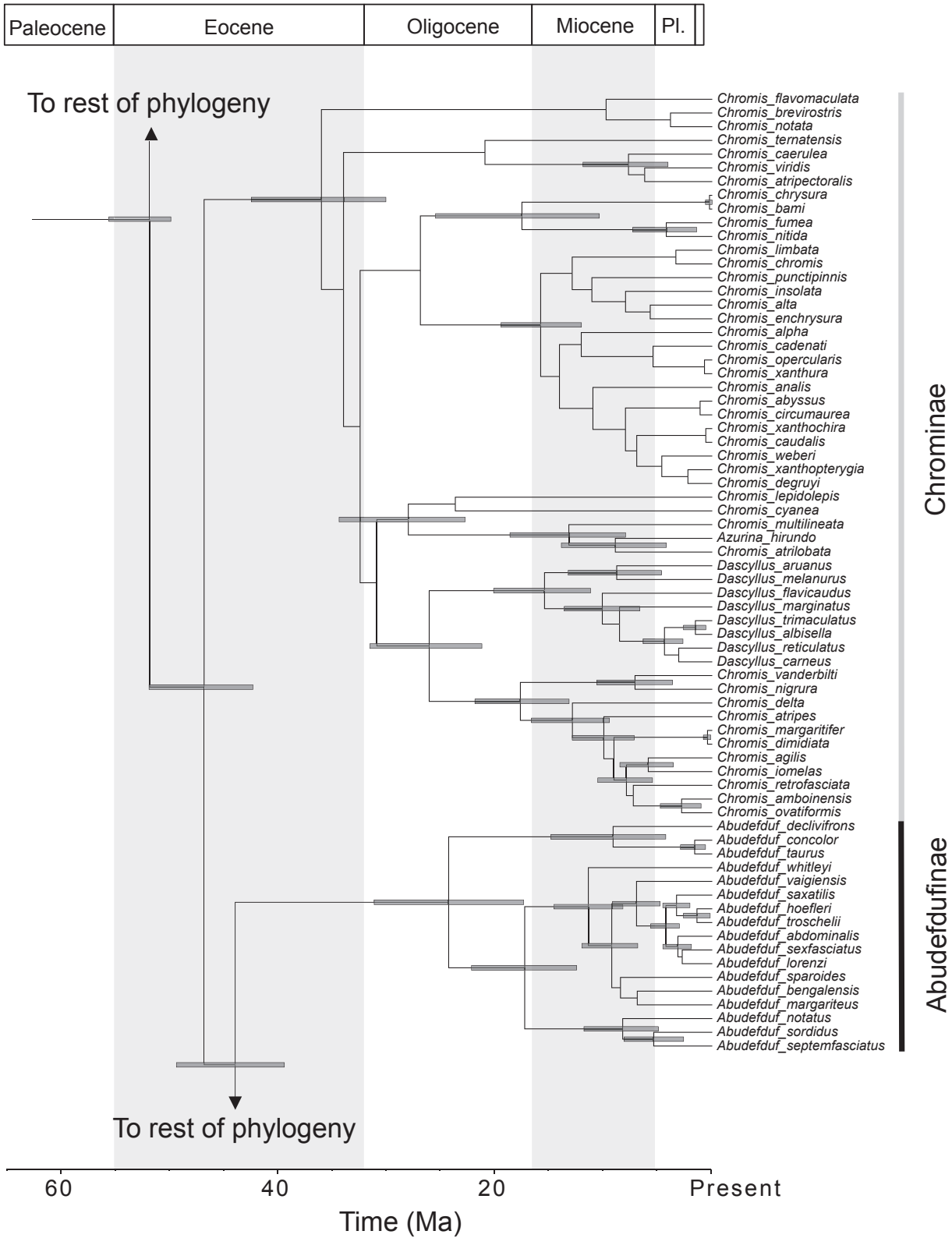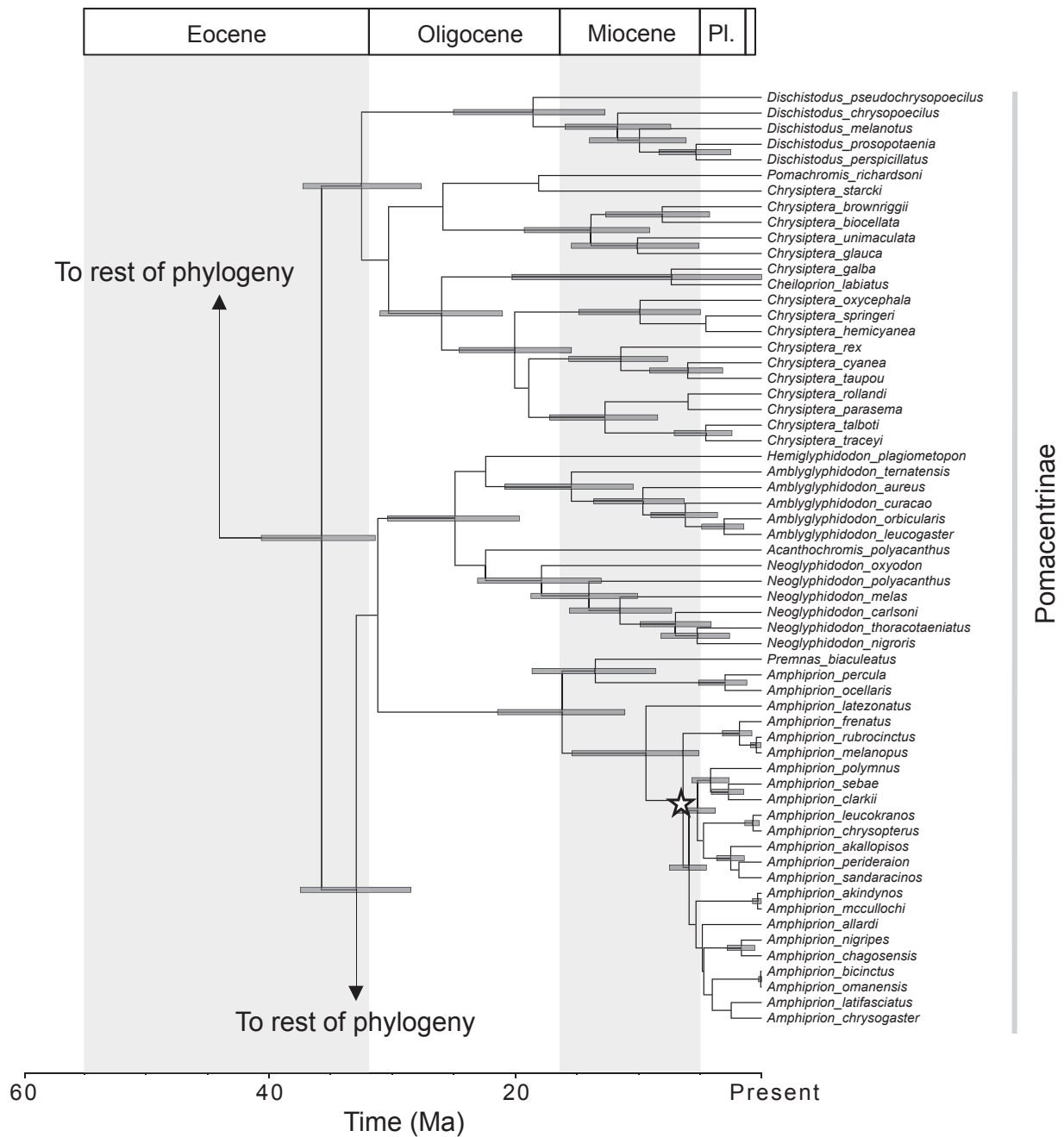
Figure 2 (*Continued*)

Figure 2 (*Continued*)

(pure-birth, birth-death, and density-dependent models) failed to find strong support for one model over the others (table 1). The two density-dependent models, DDL and DDX, together accounted for more than 70% of the Akaike weight, although again, this may be a consequence of incomplete sampling. Consequently, we are unable to validate a pattern of high diversification rate early in the his-

tory of the clade even though γ itself is highly negative. MEDUSA revealed strong support for a rate shift at the node grouping crown *Amphiprion* species, that is, excluding *Premnas biaculeatus*, *Amphiprion ocellaris*, *Amphiprion percula*, and *Amphiprion latezonatus* (ΔAIC = 7.61, fig. 2). The net diversification rate for this clade (0.259 lineages/ million years) was over 2.5 times higher than the back-
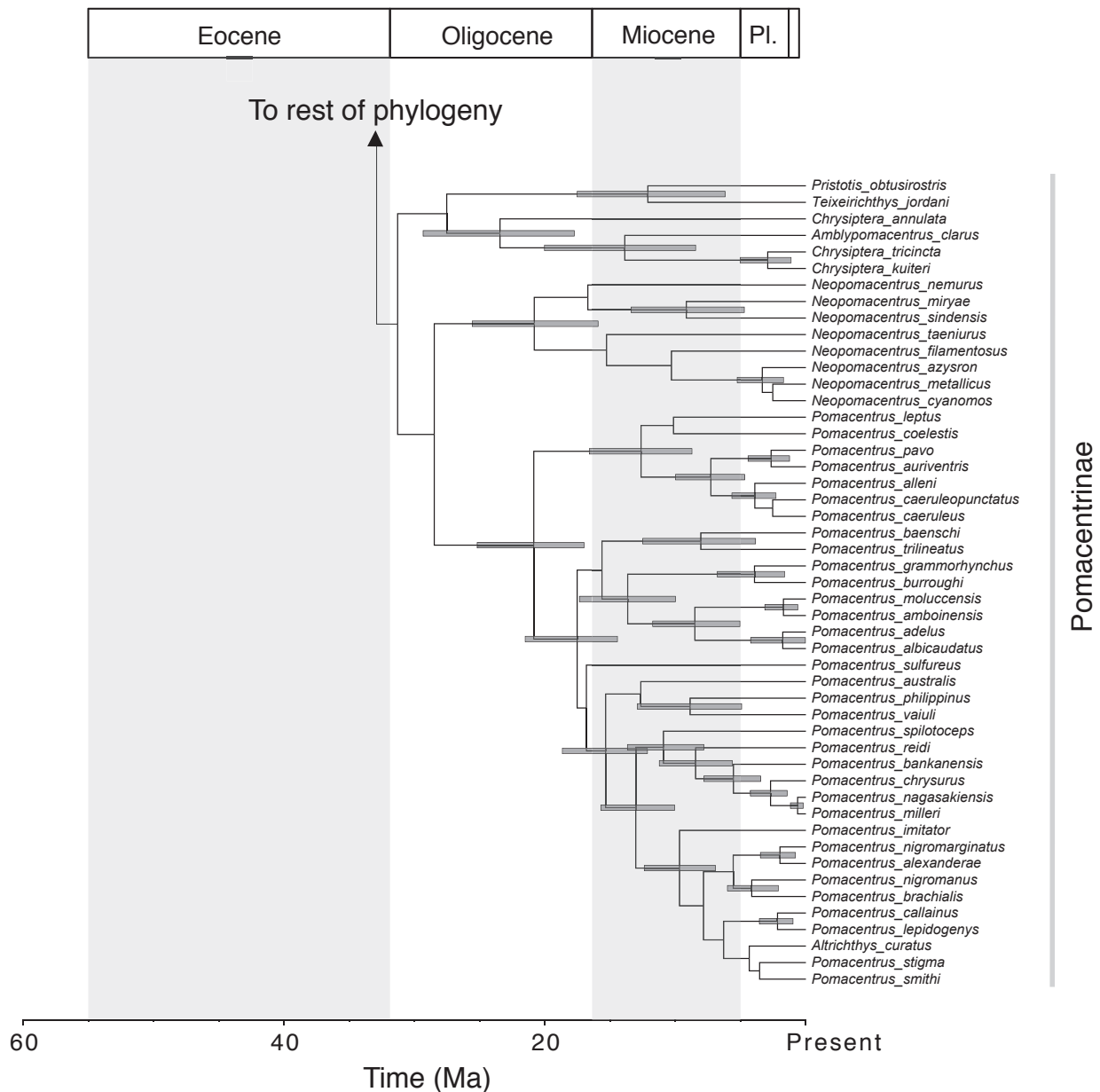
Figure 2 (*Continued*)

ground rate of diversification in other damselfishes (0.097 lineages/million years).

Major Axes of Oral Jaw Shapes Variation

Relative warps calculated from the morphological (i.e., 129 species) and phylogenetic (i.e., the 124 species included in our time-tree) data sets identified similar major axes of shape variation explaining similar amounts of variation

$(\Delta|\text{warps}_{129\,\text{species}} - \text{warps}_{124\,\text{species}}| \leq 1.5\%)$. Warps from the morphological data set are described below.

The first three relative warps explained 79.9% (RW1 = 58.8%, RW2 = 13.3%, and RW3 = 7.8%) of the total mandible shape variance. The first axis of mandible shape change described a general decrease in height of the mandible (fig. S2, available online). Damselfishes with low values of warp 1 had robust mandibles with high coronoid processes (landmarks [LMs] 2, 3) and a long symphysis (LMs 1, 12), while species with high values of warp 1

Table 1: Results from fitting diversification-process models

Model	AIC	ΔAIC	wtAIC	r	Parameter
DDL	−360.050	0	.485	.103	.103
DDX	−358.523	1.527	.226	.135	.111
Pure birth (Yule)	−358.386	1.664	.211	.082	
Birth-death	−356.386	3.664	.078	.082	.000

Note: Models are ranked from best to worst, according to Akaike Information Criterion (AIC) scores and Akaike weights (wtAIC). ΔAIC scores indicate the difference between the candidate model and the best-fitting model. Also provided are the initial speciation rate (r) and, if applicable, an additional model-specific parameter.

possessed very thin mandibles. The second warp axis described a shortening of the symphysis and a global shortening of the articulo-angular (LMs 4–9). The third warp axis described a lengthening of the ventral region of the articulo-angular (LMs 7, 9) and the retroarticular (LMs 6, 7).

For the premaxilla, the first three warps accounted for 87.6% of the overall variance (RW1 = 55.6%, RW2 = 24.6%, and RW3 = 7.4%). The first warp axis described a shortening of the dentigerous process (LMs 1, 6) and a global lengthening of the ascending process (LMs 1, 2; fig. S2). Species with low values of warp 1 possessed a premaxilla with a long ascending process and a short dentigerous process. Both processes formed an acute angle. Conversely, damselfishes with high values of warp 1 possessed a premaxilla with a relatively short ascending process and a long dentigerous process, and both processes formed an angle almost equal to 90°. The second warp axis mainly expressed a shortening of the spine of ascending process (LMs 2, 3). The third warp axis characterized a thinning of the dentigerous process (LMs 4–6).

Ecological Diversification and Model of Morphological Evolution

Based on ancestral state reconstruction under parsimony and maximum likelihood (fig. 3), farming behavior evolved at least once within the subfamily Stegastinae and three times within Pomacentrinae. The three feeding behaviors are widely distributed within Pomacentrinae, Abudefdufinae, and the clade formed by Stegastinae and the monospecific Lepidoziginae. Chrominae mainly contains zooplanktivorous species, except some *Dascyllus* species that feed on small planktonic and benthic preys. The benthic feeder and intermediate ecotypes appear 16 and 14 times, respectively, along the history of Pomacentridae (fig. 3).

The OU_T model, which allows each feeding behavior to take a separate trait optimum, was best supported for most of the shape variables (all except mandible warps 2 and 3) and body size. Support for BM and the ACDC

model was low in the majority of cases (table 2), and in all cases, the maximum likelihood estimates for the ACDC model suggested increasing rates through time rather than an early burst (table S2, available online). Taken together, these results support the hypothesis that diet differences drove oral jaw shapes diversity in damselfishes but that these traits did not diversify early in damselfish phylogeny. This finding is robust to analyses accounting for measurement error, which could mask an underlying early burst of trait evolution (app. S2, table S3, available online). Focusing on the best model for body size, OU_T, we found evidence for smaller optimal sizes in benthic feeders (SL = 76 mm) than in pelagic feeders (SL = 89 mm) and intermediate group (SL = 92 mm). Mean (i.e., the consensus shape of a trophic group) and optimal (i.e., the predicted values by OU_T model) shapes were highly consistent for both skeletal units (fig. 4).

The disparity-through-time plots of oral jaws shape strongly suggest repeated morphological radiations and convergences rather than a single burst of evolution during the early evolutionary history of damselfishes. Subclade disparity through time is higher than the median value expected under a null BM model for the majority of traits (fig. 5), indicating the morphological disparity is distributed within clades than among clades. Disparity in mandible shape shows a pattern of relative constancy through time although a burst of shape evolution occurs relatively early in the history of damselfishes (∼0.15–0.25 relative time).

Disparity and Diversification in Morphospace

Visual exploration of morphospaces reveals a strong pattern of convergence in the mandible, where all subfamilies overlap in the space defined by the three first relative warps (fig. 6). For the premaxilla, Chrominae and Stegastinae occupy distinct regions in the space defined by warps 1 and 2, while Pomacentrinae and Abudefdufinae have an intermediate position (fig. 6). Overlaps are more obvious in the space defined by warps 2 and 3.

Among the four major subclades, Pomacentrinae significantly shows the lowest level of mandible shape disparity (fig. S3, available online). The three others share similar levels, reflecting the overall level of mandible shape disparity within the family. For the premaxilla, Chrominae has the highest level of shape disparity, the Pomacentrinae intermediate and the subclades Abudefdufinae and Stegastinae + Lepidoziginae show the lowest (fig. S3). Chrominae drives the major part of premaxilla shape disparity within the family.

Patterns of oral jaw shape diversification are highly conserved among the major pomacentrid subclades. Comparison of the subspaces (defined by the first three relative
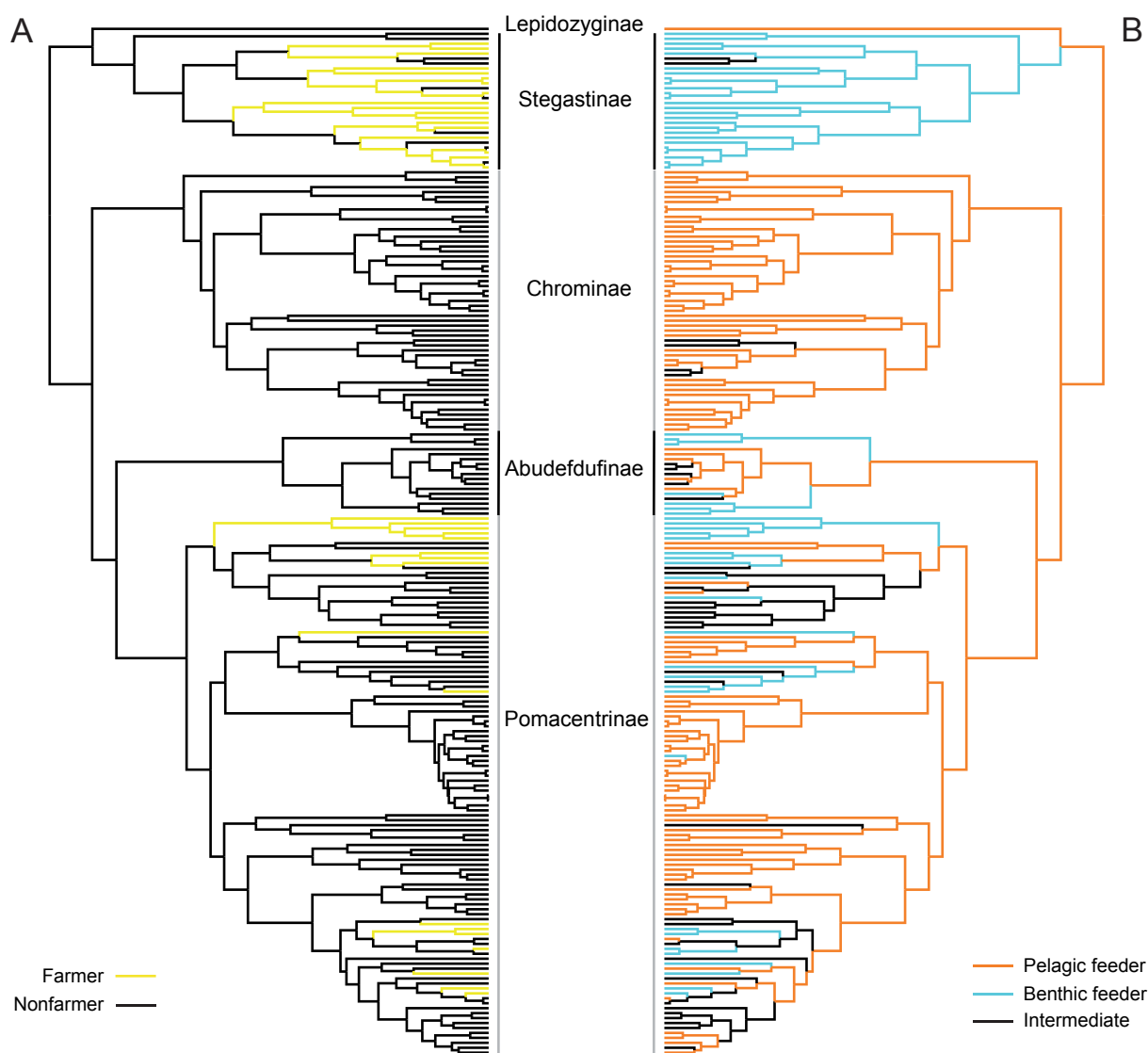
Figure 3: Consensus tree illustrating farming (*A*) and feeding behavior (*B*). Color of branches indicates inferred farming and feeding behaviors based on parsimony character mapping.

warps) occupied by each subclade revealed that most major axes of shape variation did not differ significantly across subclades (table 3), suggesting that have convergently diversified along these shape axes. For example, the angle between the subspaces of Abudefdufinae and Pomacentrinae is 73° in the mandible and thus lower than the ranges of within-hyperplane angles (89° for Abudefdufinae and 90° for Pomacentrinae). Consequently, we cannot reject the null hypothesis that Abudefdufinae and Chrominae occupy the same subspaces of morphospace, sharing the same pattern of diversification. The only exception to this was the Chrominae, which differed significantly from Po-

macentrinae (premaxilla) and Stegastinae + Lepidozyginae (mandible; table 3).

Trajectories of Morphological Evolution

A full factorial MANOVA revealed significant shape variation among subclades, ecotypes, and subclades × ecotypes interaction (table S4, available online). The results of the trajectory analysis reveal a strong pattern of repeatability in damselfish morphological diversification. For both skeletal units, the trajectory direction and the trajectory shape of morphological diversification did not dif-

Table 2: Results from fitting morphological diversification models

Morphological trait	Model	AICc	ΔAIC	wtAIC
Body size:				
logSL	OU_T	−203.7	0	.81
	OU_F	−199.6	4.10	.10
	OU	−197.9	5.75	.05
	ACDC	−197.9	5.75	.05
	BM	−135.7	68.05	<.1
Mandible:				
Warp 1	OU_T	−221.2	0	.9905
	OU_F	−211.8	9.38	.0091
	OU	−204.2	16.99	.0002
	ACDC	−204.2	16.99	.0002
	BM	−177.5	43.64	<.1
Warp 2	OU	−360	0	.45
	OU_F	−359.1	.88	.28
	OU_T	−358.9	1.08	.26
	ACDC	−343.1	16.86	<.1
	BM	−274.6	85.4	<.1
Warp 3	OU	−431.4	0	.3977
	ACDC	−431.3	.08	.3827
	OU_F	−429.6	1.83	.1596
	OU_T	−427.6	3.78	.06
	BM	−388.3	43.08	<.1
Premaxilla:				
Warp 1	OU_T	−221.7	0	.871
	OU_F	−217.2	4.48	.0929
	OU	−215.3	6.37	.0361
	ACDC	−195.7	25.99	<.1
	BM	−119.3	102.38	<.1
Warp 2	OU_T	−345.5	0	.9839
	OU_F	−337.3	8.24	.016
	OU	−327.1	18.37	.0001
	ACDC	−308.8	36.68	<.1
	BM	−235.9	109.59	<.1
Warp 3	OU_T	−469.9	0	.3724
	OU	−469.7	.22	.3337
	OU_F	−468.4	1.59	.1683
	ACDC	−467.8	2.17	.1256
	BM	−412.9	57.05	<.1

Note: For each studied morphological trait (body size and jaws relative warps), the models are ranked from best to worst, according to AICc (small-sample corrected Akaike Information Criterion) scores and Akaike weights (wtAIC). ΔAIC scores indicate the difference between the candidate model and the best-fitting model. Refer to text for model description.

fer significantly among the three major subclades (table 4). Only trajectory size varied between some subclades. The magnitude of morphological variation differed between Pomacentrinae and the group Stegastinae + Lepidozigynae + Chrominae for each unit. Magnitude differed between Abudefdufinae and Pomacentrinae for the mandible as well.

Discussion

Replicated Ecomorphological Radiation across Damselfishes

Our results provide strong evidence that repeated ecological radiations have characterized the diversification of damselfishes. Cooper and Westneat (2009) noted that the three pomacentrid trophic groups evolved repeatedly across the phylogenetic tree. Using a larger taxonomic sampling, our analyses provide a firm quantitative validation of this hypothesis, and we demonstrate the adaptive basis for these convergences by combining ecological data and quantitative morphometric analyses. Damselfish diversification is not consistent with the predictions of a single adaptive radiation with an early burst of diversification. The tempo of cladogenesis does not correspond to evolutionary models expressing a rapid initial diversification followed by a temporal slowdown in rate (table 1). Rather, our results agree with the recent study of Cowman and Bellwood (2011), revealing a relatively constant rate of cladogenesis through time in damselfishes. Only the crown species of the genus *Amphiprion* (i.e., most of the clownfishes) show a higher rate of lineage diversification than all other Pomacentridae (fig. 2). Symbioses with sea anemones (Allen 1972) could be a key innovation that allowed invasion of a new adaptive zone that has promoted speciation in this clade (Heard and Hauser 1995). It is possible that damselfishes did undergo an early adaptive radiation but high levels of turnover have since erased the signal of early lineage diversification in pomacentrid phylogeny (Quental and Marshall 2009, 2010). If this were the case, however, we would still expect to find some signal of early, rapid evolution in our ecomorphological traits (Slater et al. 2010).

We found an overwhelming signature of convergence in the phenotypic traits of damselfishes. The major subclades present a similar level of morphological disparity for each unit of the oral jaws (fig. S3). Furthermore, the main ecomorphological axes, along which the subclades diversify, are highly conserved within the family (table 3). These results are strengthened by the comparison of phenotypic trajectories, which highly suggests the repeatability of the pattern of morphological evolution (parameters of shape and orientation; table 4). Perhaps most strikingly, however, morphological disparity-through-time analysis revealed that phenotypic variation was partitioned within, rather than among clades, especially for the mandible and the premaxilla shape (fig. 5). This type of approach has typically been used to document the signal of adaptive radiation where diversity is partitioned among clades leading to negative morphological disparity index values (e.g., Harmon et al. 2003; Slater et al. 2010; Dornburg et al. 2011). The strong, opposing signal and positive MDI values recovered in our data are therefore particularly inter-
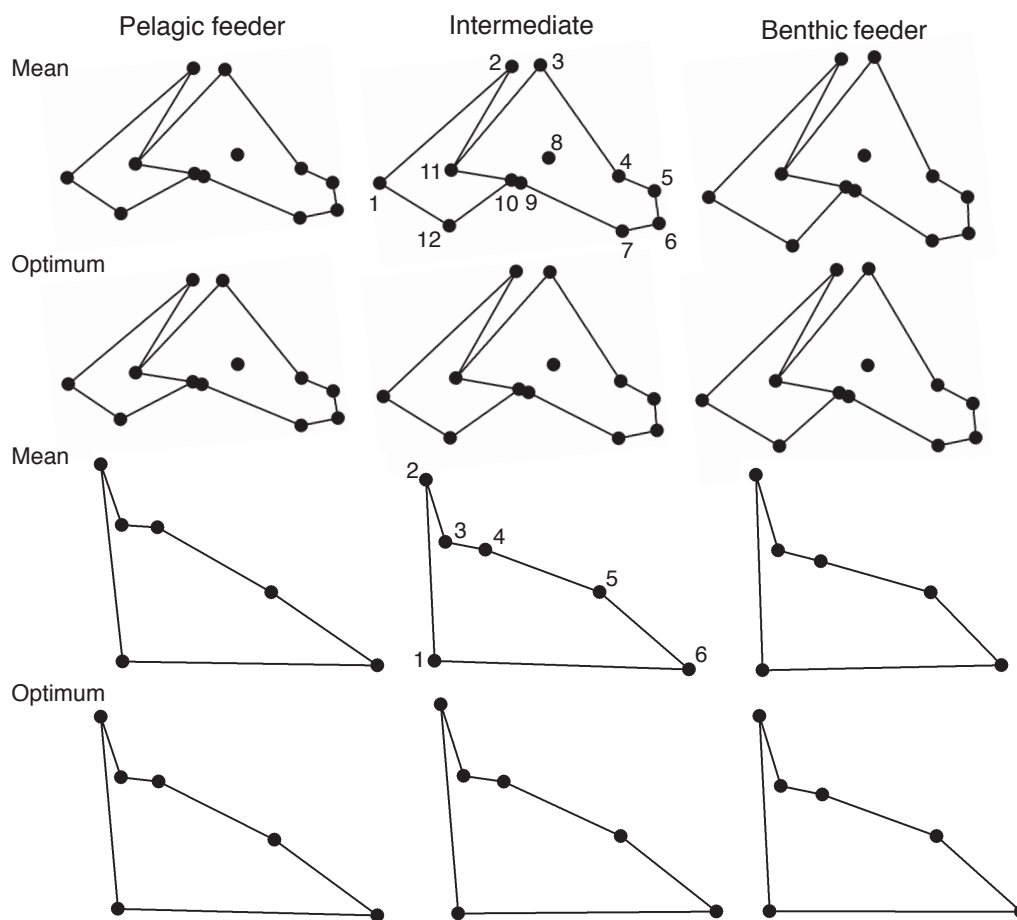
Figure 4: Illustration of mean shapes of each trophic group and optimal shapes predicted by OU_T models. Predicted warp values by OU_T models are provided in table S2, available online. Numbers refer to landmarks illustrated in figure 1.

esting and reveal a pronounced pattern of convergence in ecomorphological traits.

Diet clearly appears to be a major driving force of morphological evolution in damselfishes (Emery 1973; Gluckmann and Vandewalle 1998; Frédérich et al. 2008; Cooper and Westneat 2009; Aguilar-Medrano et al. 2011; Frédérich and Vandewalle 2011). The mapping of dietary behavior onto our phylogeny reveals ecological convergence in each major pomacentrid radiation (fig. 3). We further found strong support for different optimal oral jaw shapes and body size in damselfishes that have different trophic strategies (OU_T; table 2), demonstrating that a part of the morphological diversity in Pomacentridae was shaped by constrained evolution. Oral jaw shape varies between two extremes that typify the two most distinct functional groups: (1) the pelagic feeders catching their prey by ram/suction feeding and (2) the benthic feeders grazing filamentous algae and/or biting fixed small invertebrates. Between these ecotypes, intermediate forms include species

feeding on variable amounts of zooplankton, filamentous algae, and small benthic invertebrates. We found little support for the OU_F model (table 2), revealing that farming behavior did not constrain the diversification of the studied traits. Farming could be a behavioral adaptation driving physiological or other morpho-functional variation not studied here (e.g., adductor mandibulae muscle masses). However this unique ecological behavior certainly warrants further attention in evolutionary studies of damselfishes. In the context of the traits studied here, the evolutionary history of Pomacentridae exemplifies a type of iterative adaptive radiation where distinct selective ecomorphological peaks lead to repeated, predictable evolutionary patterns (Losos 2011).

Repeated patterns of evolutionary change in phenotypic traits are commonly regarded as evidence of adaptation under common selection pressures in similar environments (Losos 2011). This kind of iterative radiation has been demonstrated for organisms that colonize new islands
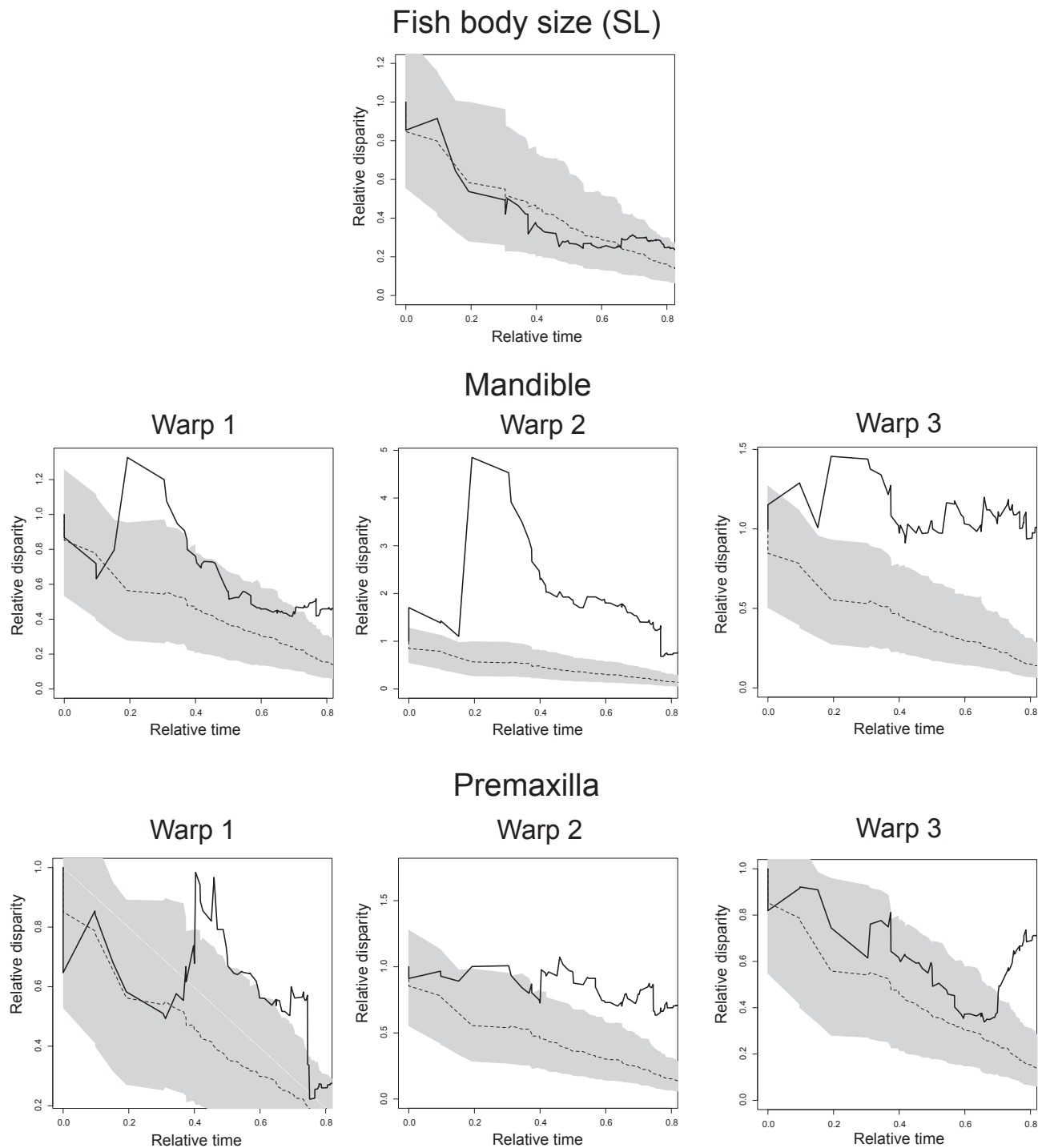
Fish body size (SL)

Mandible

Warp 1

Warp 2

Warp 3

Premaxilla

Warp 1

Warp 2

Warp 3

Figure 5: Disparity-through-time plots calculated individually for each morphological trait. The MDI statistic (relative disparity) was calculated over 80% of the time tree (relative time ranges 0–0.8). Standard length (SL) refers to fish body size (maximum SL), and warp 1, warp 2, and warp 3 are the main axes of shape variation for the mandible and the premaxilla. The biological significance of warps is described in "Results." The dotted line indicates the relative disparity expectation according to a Brownian model of evolution based on 10,000 simulations; the solid line is data from the study. The gray shaded area indicates the 95% range for the simulated data.

Mandible
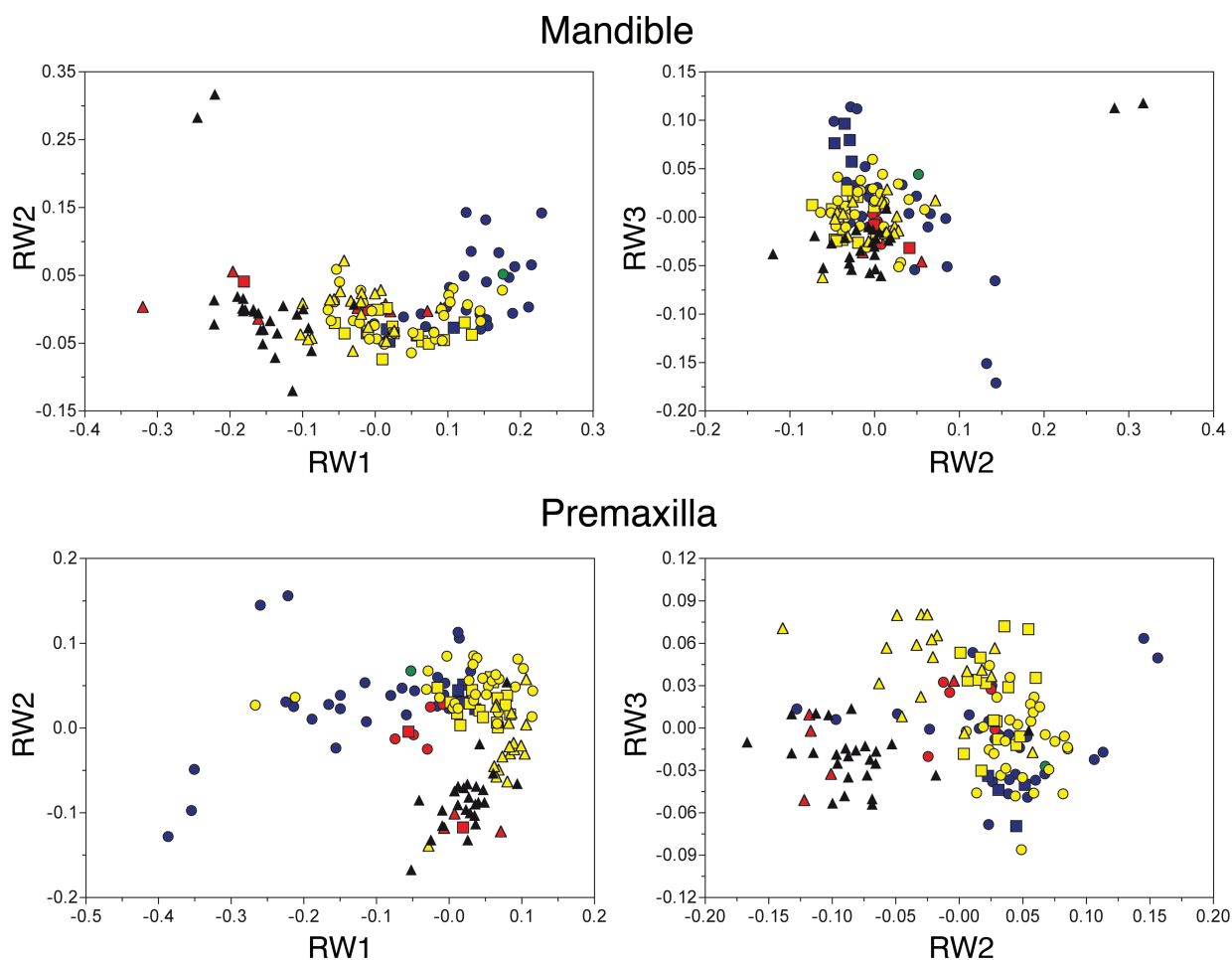
Premaxilla

Figure 6: Morphospace visualizations for the mandible and the premaxilla. The three first relative warps (RWs) are illustrated for both skeletal units. The subfamiles and the trophic groups are differentiated by color codes (black = Stegastinae, green = Lepidozyginae, red = Abudefdufinae, blue = Chrominae, yellow = Pomacentrinae) and icons (circle = pelagic feeders, square = intermediate, triangle = benthic feeders), respectively.

or lakes (Losos and Ricklefs 2009), but damselfishes and Antartic notothenioids (Rutschmann et al. 2011) illustrate the first cases of repeated adaptive radiations occurring in the oceans. In coral reef ecosystems, repeated radiations might be expected during events of regionalization or geographic expansion allowing the colonization of new reefs. It is well known that some reef regionalization was induced by the appearance of physical barriers such as the mid-Atlantic barrier (60–80 Ma), the Red Sea land bridge (terminal Tethys event, 12–18 Ma), the Isthmus of Panama (3–3.5 Ma), or by restricted surface water exchanges between Indian and Pacific basins during the late Miocene (Kennett et al. 1985; Floeter et al. 2008). The appearance of volcanic islands also provided unoccupied areas for coral reef fishes. All of these events could have influenced

the radiation of damselfishes, creating novel ecological opportunity for convergent radiations. Even if the major subclades did arise during periods of isolation, the occurrence of subsequent dispersal led to major subclades being now all overlapping in tropical and subtropical waters (Cooper 2008).

Various constraints may lead to convergences and repeated radiations (Losos 2011). Factors such as competition could be viewed as an external constraint. Developmental processes, pleiotropic effects, morphological integration, or peculiar morphological characters can all provide sources of internal constraint leading to a limited phenotypic repertoire in evolutionary radiations (Losos 2011). Here, we cannot yet say whether a highly competitive environment or a damselfish-specific anatomical con-

Table 3: Comparisons of the patterns of shape disparity among subclades

Element	Comparison	Between clades (95% CI)	Within clade 1	Within clade 2
Mandible	S+L − A	82.9 (74.6–106.3)	82.1	89.9
	S+L − C	**101.6 (93.0–113.6)**	**68.9**	**66.1**
	S+L − P	63.2 (60.4–102.9)	65.5	89.4
	A − C	96.7 (84.4–106.6)	89.0	85.1
	A − P	72.5 (66.5–101.4)	88.7	89.8
	C − P	94.1 (68.9–105.5)	66.9	89.1
	S+L+A+C − P	66.7 (57.7–97.2)	51.4	88.7
Premaxilla	S+L − A	55.4 (43.6–99.5)	86.4	79.4
	S+L − C	67.8 (59.0–95.3)	80.9	31.4
	S+L − P	54.3 (43.3–79.7)	84.0	30.9
	A − C	75.6 (50.0–90.8)	78.3	76.5
	A − P	65.5 (58.2–91.3)	77.2	44.7
	C − P	**68.7 (60.1–83.5)**	**35.3**	**29.0**
	S+L+A+C − P	40.7 (36.9–65.2)	71.2	22.4

Note: Results are obtained by bootstrapping procedures ($N = 1,600$) using SpaceAngle. Angles between hyperplanes—that is, the subspaces defined by the first three relative warps—are in decimal degrees. The angle between hyperplanes is considered significant (bold) if it exceeds the bootstrapped within-group variance at 95% confidence. Abbreviations S, L, A, C, and P refer to Stegastinae, Lepidoziginae, Abudefdufinae, Chrominae, and Pomacentrinae, respectively. CI = confidence interval.

straint led to such repeated radiations, but these hypotheses will need to be tested.

Single versus Replicated Radiations in Macroevolutionary Studies

Adaptive radiation is frequently invoked to explain the existence of strikingly diverse clades (Olson and Arroyo-Santos 2009; Lieberman 2012) and macroevolutionary research over the past decade has been dominated by a search for its signature in comparative data. Recent work (e.g., Harmon et al. 2010) has suggested that the telltale signals of adaptive radiation (early bursts of lineage diversification and trait evolution: Simpson 1944; Schluter 2000; Gavrilets and Losos 2009) may in fact be rare in comparative data. One possible explanation for the absence of unambiguous signs of a single adaptive radiation is that at larger phylogenetic scales, iterative adaptive radiations is not uncommon and that these repeated evolutionary excursions into a limited set of niches erode the signal of more local-scale radiations. Convergent morphological evolution, an expected outcome to similar functional demands, is a well-documented evolutionary phenomenon (Winemiller et al. 1995; Ruber et al. 1999; Stayton 2006; Revell et al. 2007; Herrel et al. 2008; Hulsey et al. 2008) but the interplay between convergence and adaptive radiation has not been widely considered (Losos 2010). Instead, most adaptive radiation examples emphasize the diversity of new niches

Table 4: Comparisons of the trajectories of morphological evolution among subclades

Element, comparison	$MD_{1,2}$	P_{size}	$\theta_{1,2}$	P_{θ}	D_{shape}	P_{shape}
Mandible:						
S+L+C − A	.0224	.7886	158.1102	.4141	.2906	.4650
S+L+C − P	**.3161**	**.0001**	23.0453	.1988	.1803	.5681
A − P	**.2937**	**.0026**	158.5691	.4518	.4064	.2382
Premaxilla:						
S+L+C − A	.1141	.1034	40.3096	.7555	.2214	.6444
S+L+C − P	**.1947**	**.0001**	45.2878	.7975	.3375	.1860
A − P	.0805	.2502	51.6769	.4013	.1464	.8347

Note: Statistical assessment of differences in the attributes of trajectories: size ($MD_{1,2}$), orientation ($\theta_{1,2}$), shape (D_{shape}). Bold values highlight significant differences. Observed significance levels (P values) were obtained from 10,000 random permutations. Abbreviations S, L, A, C, and P refer to Stegastinae, Lepidoziginae, Abudefdufinae, Chrominae, and Pomacentrinae, respectively. Refer to the text for the reasons for grouping some subfamilies.

that are made available to the radiating lineage however we might expect that the extent of ecological opportunity will vary depending with the traits possessed by the lineage and the external environment. However the limits or constraints on ecological opportunity clearly play an important role in shaping the outcomes of adaptive radiation.

Replicated radiation and convergence appear to constitute some of the best-known cases of adaptive radiation. The *Anolis* radiation on Caribbean islands displays strong patterns of diversity-dependent evolution within islands while appearing highly convergent when considered as a whole (Losos 2009; Mahler et al. 2010; Rabosky and Glor 2010). The cichlids in individual African rift lakes beautifully illustrate the concept of explosive adaptive radiation, but taken as a whole, the radiations constitute an example of repeated radiations (Kocher 2004; Genner and Turner 2005; Salzburger et al. 2005; Seehausen 2006). Replicate divergences of phytophagous insects across host plants (e.g., Stireman et al. 2005) and of benthic and limnetic forms of sticklebacks in Holarctic postglacial lakes (Schluter 2000) further suggest that a pattern of repeated radiations has the potential to explain a significant proportion of diversity. Future studies which explicitly consider the possibility of replicated radiations could help reconcile the apparent inconsistencies between empirical patterns of diversification in diverse groups (Harmon et al. 2010; Slater et al. 2010; Dornburg et al. 2011) and macroevolutionary theory, and reveal the importance of constraint in shaping diversity patterns across the tree of life (Gavrilets and Losos 2009).

Conclusion

Coral reef fishes represent one of the most dramatically diverse assemblages of vertebrates on the planet, but our understanding of their mode of diversification remains limited. Our study illustrates that iterative ecological diversification, rather than one single adaptive radiation, provides a better explanation for the diversity of extant damselfishes. Our results are consistent with recent findings that suggest that the classical concept of adaptive radiation with an early burst of lineage and phenotypic diversification (Simpson 1944; Schluter 2000) may have limited power to explain the morphological diversity in higher taxa. To our knowledge, damselfishes illustrate one of the first demonstrated iterative ecological radiations occurring in oceans. Regionalization of coral reefs, competition, and functional constraints may be causal factors underlying this evolutionary pattern.

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Damselfish (*Abudefduf sordidus*) in the Red Sea. Photograph by Bruno Frédérich.