Colour and shape evolution reflect ecological specialisation in Pomacentridae

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Abstract

How ecological pressures shape phenotypic traits such as colouration and morphology is a fundamental question in evolutionary biology. Colouration often mediates functions such as communication, camouflage, and species recognition, while morphological traits influence locomotion, feeding, and habitat use. In reef fishes, both traits exhibit striking diversity, frequently shaped by ecological interactions. The damselfish family (Pomacentridae) exemplifies this variation, displaying a wide range of colouration, morphology, and ecological strategies, including dietary specialisation, mutualistic behaviours, and freshwater tolerance. In this study, we examined 342 Pomacentridae species using an integrative framework that combined colouration and morphometric analyses, ancestral state reconstructions of habitat, diet, and symbiosis, and Phylogenetic Comparative Methods (PCMs). Our goal was to assess how ecological factors have influenced the evolution of phenotypic traits and to identify patterns of convergence linked to ecological specialisation. We found repeated convergent evolution in both colouration and body shape, often associated with similar ecological pressures. Species with specialised diets exhibited consistent morphological adaptations, underscoring the role of trophic ecology in shaping body form. Additionally, we observed a strong association between habitat type and dominant colouration patterns, consistent with a camouflage function. These findings clarify the evolutionary processes driving phenotypic diversity in damselfishes and highlight the importance of ecological adaptation in shaping both morphology and colouration. Our work contributes to a broader understanding of how form and function evolve in response to ecological pressures in coral reef ecosystems.

Keywords: pomacentridae, evolution, coloration, morphology, convergence, PCM, habitat, symbiosis, diet

Introduction

The extraordinary diversity of reef fishes reflects a complex interplay between phenotypic traits and the ecological roles species occupy within their environments [1, 2]. Across reef ecosystems, morphology and colouration —two fundamental axes of phenotypic variation— evolve in response to a mosaic of biotic interactions and abiotic constraints, driving adaptations related to habitat use, diet, and social behaviour [3–11]. Although the functional significance of morphology and colouration has been extensively documented in reef fishes, these traits are frequently studied independently. This fragmented approach limits our understanding of whether and how morphology and colouration coevolve in response to shared ecological pressures, leaving fundamental questions about the interconnected dynamics of phenotypic evolution unresolved.

Functional hypotheses for morphological traits are relatively straightforward, as these often show clear, measurable links to ecological performance —for example, jaw architecture relates to foraging behaviour, and body shape to swimming efficiency and habitat use [e.g. 3, 4, 12]. In contrast, formulating functional hypotheses for colour patterns is more complex. Reef fishes display striking visual diversity shaped by mimicry, camouflage, social signalling, and sensory constraints [8, 13–19]. Caribbean hamlets (*Hypoplectrus* spp.) exemplify this interplay, with colour morphs driven by assortative mating and aggressive mimicry, illustrating how sexual and ecological selection act in concert [20, 21]. Caudo-rostral stripes in shoaling fishes confuse predators [22], butterflyfish (Chaetodon spp.) stripe patterns correlate with habitat and diet [23], red fluorescence in benthic or predatory species provides background-matching camouflage [8], and diverse markings facilitate species recognition across many reef fish clades [24, 25]. However, these functions are often context-dependent and may vary across different environments and species [26].

Moreover, the study of colour patterns has often relied on simplified categorizations, such as manual scoring of discrete patterns [e.g., stripes, bars, eyespots) 10]. While these simplified approaches have provided valuable insights [e.g 23, 26], they may overlook the ecological significance of more complex or subtle colour variations. Such oversimplification can bias research towards certain well-studied functional roles —such as species recognition and mimicry [e.g. 8, 16, 18]— potentially overlooking other important ecological functions encoded by more complex or continuous variation in colour patterns, including habitat associations and dietary specializations [e.g. 27, 28].

The Pomacentridae —a diverse family of damselfishes and clownfishes— offers a compelling model to examine the interplay between morphology, coloration and ecological specialization. Encompassing over 400 species across 29 genera [29], pomacentrids rank among the most species-rich reef fish families and play key functional roles in coral reef ecosystems [30–32]. They occupy a wide range of habitats —from pelagic waters to benthic reef crevices— and depths, with some species even adapted to freshwater environments [33]. Their ecological breadth spans a gradient of dietary strategies, from plankton-feeding to algae-farming herbivory [33, 34]. Their behaviors range from aggressively territorial species such as the three-spot damselfish [*Dascyllus trimaculatus;* 35–39] to more cooperative forms, such as the longfin damselfish (*Stegastes diencaeus*) which engages in mutualistic interactions with shrimp farmers [14]. These ecological differences manifest in repeatable shifts in jaw morphology, habitat use, and body shape —traits often organized into ecotypes that combine feeding and microhabitat specialization [40, 41]. Pomacentrids also exhibit exceptional variation in body colouration, ranging from uniform hues and sharp bicolour gradients to UV-reflective markings, bands, stripes, and eyespots. This rich visual diversity has long intrigued researchers seeking to understand the evolutionary and ecological drivers of such elaborate phenotypic traits.

Recent studies have clarified pomacentrid phylogeny and diversification [34, 42, 43], and revealed widespread morphological convergence, with repeated transitions among ecotypes driving iterative evolution of jaw and body forms [4, 44]. These shifts reflect adaptation to diverse reef habitats and diets, underscoring the tight interplay between form, function, and ecology within Pomacentridae [45]. In contrast, whether coloration evolves along similar trajectories remains unclear. Although convergent colour patterns in clownfishes, linked to mutualistic interactions with sea anemones, provide compelling examples of ecological influence [11], these findings derive from a limited subset of the family. This raises a broader question: is colour convergence a unique feature of clownfishes, or —like morphological traits— does it reflect repeated evolutionary responses to shared ecological pressures across the Pomacentridae? Furthermore, do colour and morphology evolve independently under distinct constraints, or do they co-evolve as integrated traits shaped by common ecological factors?

Emerging ecological evidence suggests that coloration may indeed mirror patterns seen in morphology. Pomacentrid species occupying similar reef habitats—such as shallow, wave-exposed areas versus deeper, sheltered lagoons—often exhibit convergent colouration patterns that may function in camouflage, social signaling, or predator deterrence [46, 47]. Species-level hue diversity has also been linked to the richness of particular reef fish families and visual environments [48, 49], hinting that colour traits could evolve in response to habitat-specific visual constraints. Yet, while macroevolutionary studies have rigorously demonstrated morphological convergence across ecotypes [4, 34], no equivalent comparative framework has been applied to colour pattern evolution. This gap limits our understanding of whether colouration follows predictable evolutionary paths shaped by ecology, or reflects a more labile, lineage-specific dynamic within the family.

In this study, we test this question using a comparative framework integrating colour pattern, body morphology, and ecological data across 342 species of Pomacentridae (314 damselfishes and 28 clownfishes). We combine landmark-based geometric morphometrics with multivariate quantification of colour patterns from standardized images, reconstruct the biogeographic and ecological histories of species using data on diet, habitat, and symbiotic associations, and apply phylogenetic comparative methods to infer evolutionary dynamics. Our aim is to assess whether repeated transitions among ecological states have shaped phenotypic evolution along two axes—morphology and colouration—and whether these axes respond in parallel or independently to ecological and biogeographic filters. By integrating colour, morphology, and ecology across the Pomacentridae phylogeny, we uncover distinct evolutionary dynamics that reveal how ecological specialization repeatedly structures phenotypic diversification.

Results

Patterns of colour and morphological diversity

The first eight principal components (PC1–PC8) from the PCA of 1,183 Procrustes-aligned colour pattern images (342 pomacentrid species) explained 50.5% of total colour variation. PC1 (18.8%) represented a gradient from light to dark pigmentation, while PC2 (13.2%) separated reddish from bluish hues (Figure 2a). PC3 (4.4%) captured differences between body and edge pigmentation; PC4 (3.8%) reflected dorsoventral contrasts (Figure 2b). PCs 5–8 (2.2–3.0%) described more localized features such as lateral asymmetry (PC5), dorsoventral modularity (PC6), vertical segmentation (PC7), and brightness/contrast variation (PC8; Figure 2c,d).

Morphometric PCA on 11 standardized traits from the same species set captured 92.4% of total shape variation within the first eight PCs (Figure 3). PC1 (25.7%) reflected body elongation and peduncle depth, while PC2 (21.5%) described variation in head size and snout angle and length. PCs 3–8 described decreasingly (from 10.9% to 5.1%) subtle features, such as eye orientation, snout and fin proportions, and caudal peduncle shape.

Species-averaged PERMANOVAs revealed strong ecological correlates of colouration. Habitat explained the most variation ($R^2 = 0.450$, $p = 0.001$), followed by diet ($R^2 = 0.231$, $p = 0.003$) and symbiosis ($R^2 = 0.164$, $p = 0.020$). In contrast, ecological effects on morphometrics were weak and non-significant, with residuals explaining >97% of shape variation.

Permutational multivariate analysis of variance (PERMANOVA) on individual-level data showed that species

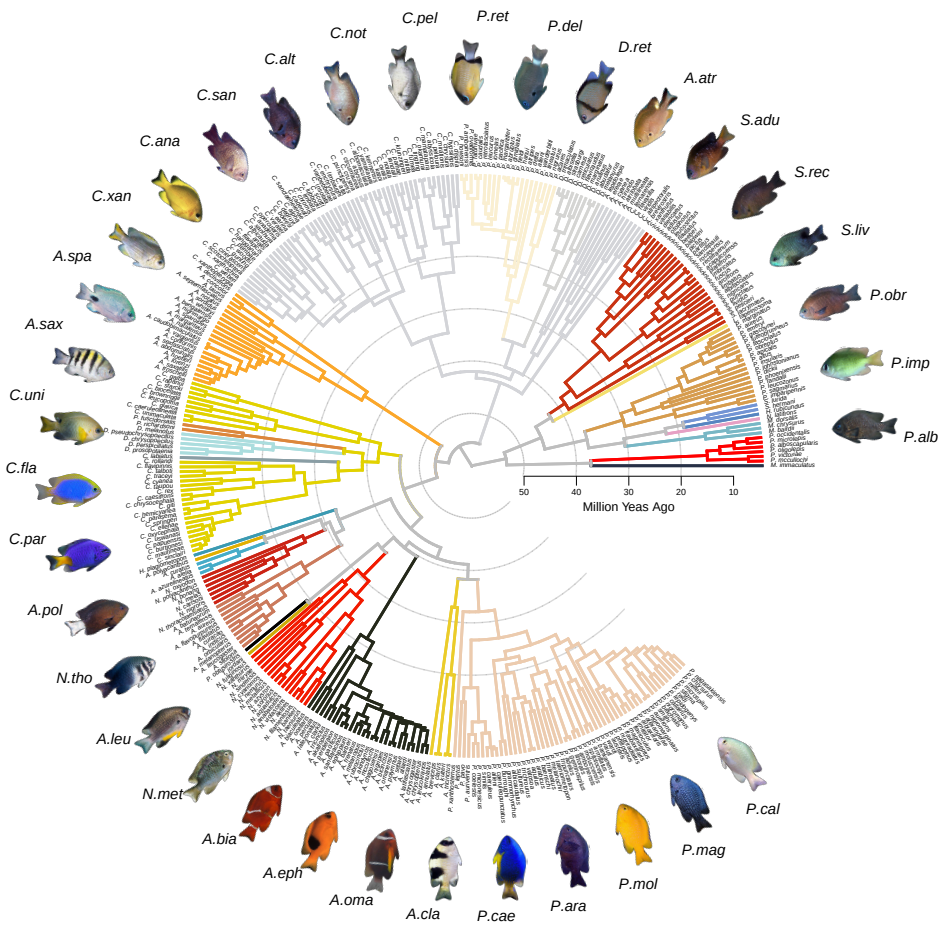

Fig. 1: Circular phylogenetic tree sourced from McCord *et al.* (2021), with branches colour-coded to distinguish species by genus. Procrustes-transformed images of 34 out of the 342 species utilized in this study are displayed in the outer margin encircling the tree. Species names are abbreviated using the initial of the genus followed by the first three characters of the species name. Horizontal scale depicts time in million years before the present (Mya).

identity explained a large share of variation in colour patterns ($R^2 = 0.715$, $F = 5.86$, $p = 0.001$), indicating strong interspecific differentiation. In contrast, variation in morphometric traits was not significantly explained by species identity ($R^2 = 0.319$, $F = 1.13$, $p = 0.291$), implying that morphological differences among species were not consistently greater than within-species variation.

Species-averaged PERMANOVAs to the effects of ecological factors revealed that diet, habitat, and symbiosis each significantly explained interspecific variation in colouration. Habitat explained the largest proportion of variation in colouration ($R^2 = 0.450$, $F = 5.77$, $p = 0.001$), followed by diet ($R^2 = 0.231$, $F = 3.55$, $p = 0.003$) and symbiosis ($R^2 = 0.164$, $F = 2.74$, $p = 0.020$), indicating that these traits may play a significant role in shaping colouration patterns. In contrast, ecological effects on morphometrics were weak and non-significant, with residuals explaining $> 97\%$ of shape variation.

Disparity-through-time (DTT) analyses on the first eight phylogenetic principal components (PCs) for both colouration and morphology revealed consistent and significant deviations from Brownian Motion (BM) expectations (all $p < 0.001$; Extended Data Figs. 4), indicating strong temporal structuring in Pomacentrid phenotypic evolution.

Color PCs exhibited uniformly elevated within-subclade disparity, with MDI values ranging from 0.22 to 0.49, suggestive of pervasive between-subclade convergence and repeated evolution of similar colour traits. Notably, PC1, PC3, PC6, and PC8 displayed pronounced late-burst trajectories, with disparity rising sharply toward the present —often after the accumulation of most lineages— suggesting recent or ongoing diversification in colouration, potentially reflecting ecological selection for camouflage, communication, or mimicry in complex reef environments. In contrast, PC2, PC4, and PC7 showed more gradual trajectories, suggesting asynchronous divergence across chromatic axes.

Morphometric PCs also revealed significant, though more heterogeneous patterns of disparity. PC2, PC3, PC5, and PC8 showed elevated MDI values (> 0.3), consistent with recent morphological divergence and possible functional convergence. A striking exception was PC4 (MDI = 0.27), which tracked the Brownian expectation until a sudden and pronounced surge in disparity near the present ($\sim$ 5.5 million years ago), well after most lineages had already accumulated and far exceeding that of any other axis.

Ancestral reconstructions of ecological traits

Ancestral state reconstructions of diet ecotypes, habitat types and symbiotic relationships were conducted using the 'all rates different' (ARD) model showing lowest AIC score (Supplementary Figure S2). Our results indicate that the common ancestor pomacentrids had a intermediate diet, between pelagic planktivory and benthic herbivore, with higher transitions from intermediate omnivore to

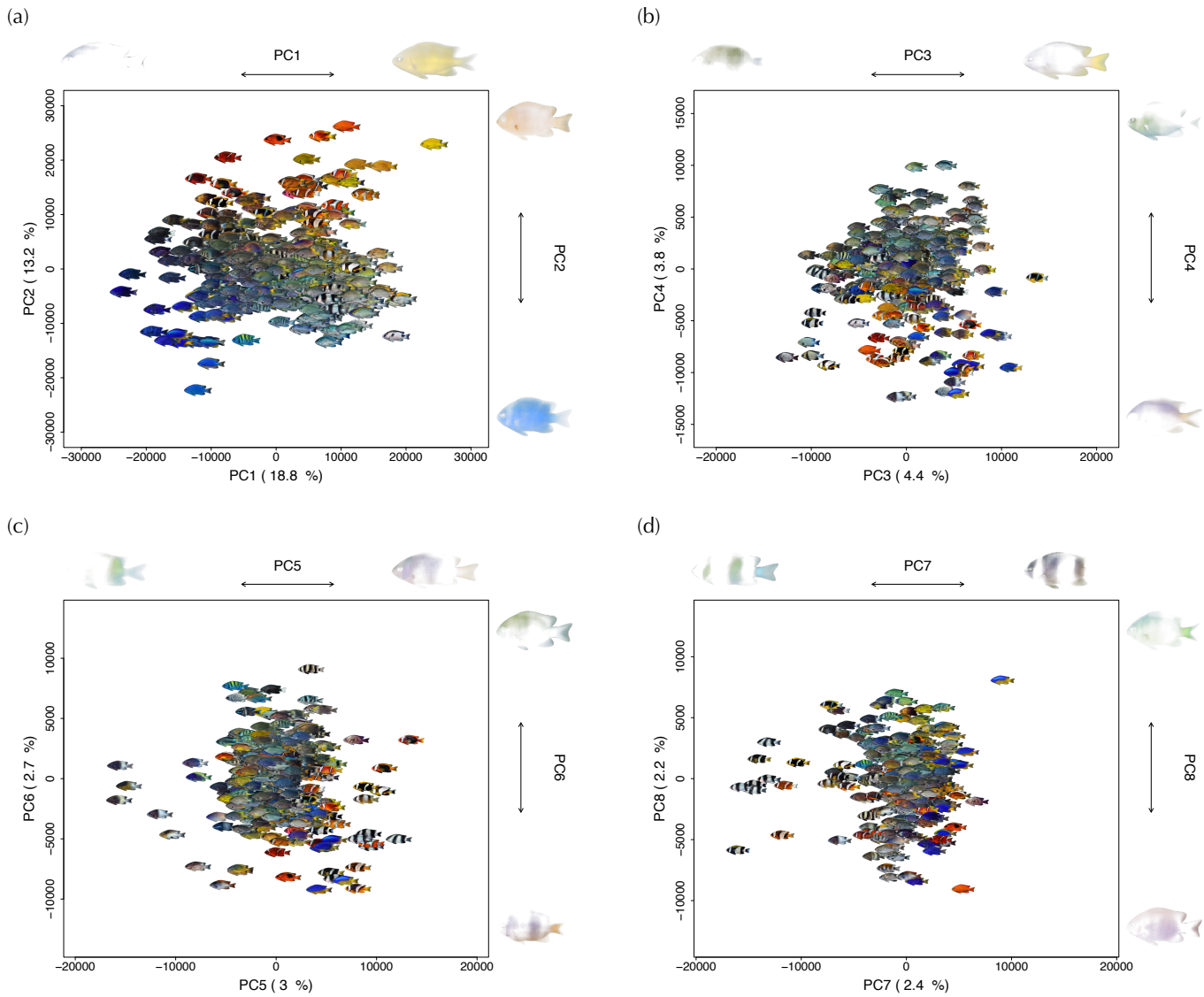

Fig. 2: Principal Component Analysis (PCA) of 1182 Procrustes-transformed images from 342 pomacentrid species. Each panel shows a different pair of Principal Components (PCs 1–8), illustrating major axes of colour pattern variation. PCA scores represent species-averaged coordinates, and the corresponding average species images are projected at those coordinates for visual interpretation. Reconstructed images on the plot margins display extreme colour pattern values along each PC axis. Pixel opacity scales with contribution to axis direction (note: white indicates zero contribution and may be indistinguishable from naturally white pattern elements). Panel (a) shows PC1 vs. PC2, (b) shows PC3 vs. PC4, (c) shows PC5 vs. PC6, and (d) shows PC7 vs. PC8. The first eight principal components (PC1–PC8) capture progressively decreasing proportions of total colour pattern variance, from 18.8% (PC1) to 2.2% (PC8), cumulatively explaining 50.5% of the total variation observed among species.

either pelagic or benthic (Supplementary Figure S3). Non-reef habitats ('seagrass-rubble-sand' class) are estimated the most probable habitat type of the common ancestor of all pomacentrids. Most transitions occur from coral reef to rocky reef followed by either rocky reef or non-reef marine habitats to coral reef habitats with few unidirectional transitions to sea anemone or freshwater habitats (Supplementary Figure S4). In respect to symbiotic relationships, most stochastic maps indicate the pomacentridae common ancestor as a comensalist symbiont with similar number of transitions from farming ("comensalistic symbiont") to symbiont-free reliationships and vice-versa (Supplementary Figure S5). Besides the transition towards mutualism at the root of the clownfish clade, two more recent transitions were estimated at the root of the sister species *Amblypomacentrus breviceps* and *A. claurus* and *Dascyllus trimaculatus* and *D. abisella*.

Ancestral reconstruction of biogeographic ranges

We used the "DEC+J" model, yielding the lowest AIC score, to carry out the ancestral biogeographic reconstruction of pomacentrids. Our results on the biogeographic reconstruction indicated a widespread origin of the clade, occurring in the Tethys Sea and the Central and

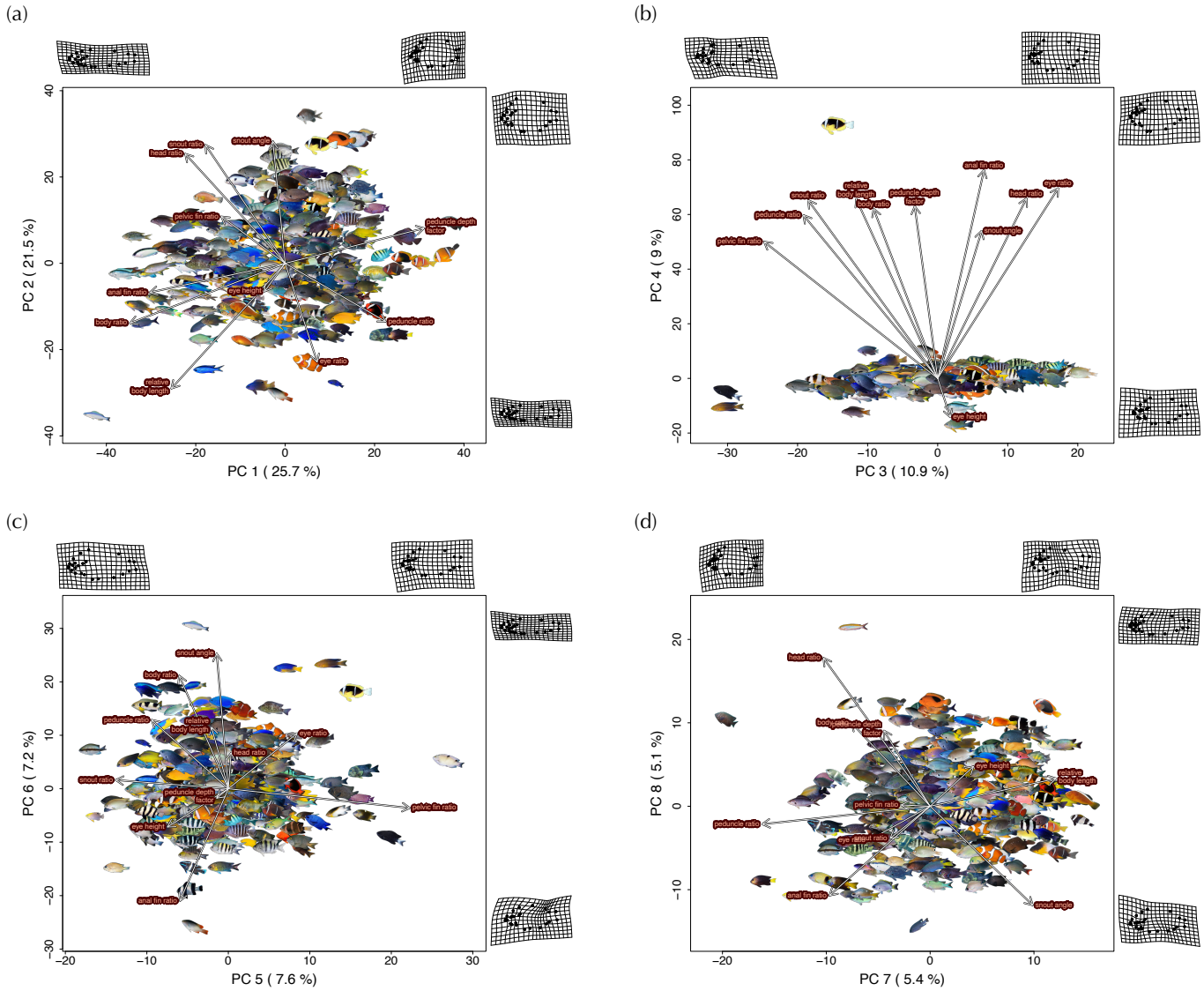

Fig. 3: Principal component morphospaces of 342 reef fish species based on 11 morphometric traits. (a) PC1 vs PC2, (b) PC3 vs PC4, (c) PC5 vs PC6, and (d) PC7 vs PC8. Each panel shows the relative position of species in morphospace with raw fish images (background removed) plotted in place of points to enhance visual interpretation of body shape variation. White arrows indicate eigenvector loadings for each trait, with arrow length denoting the relative contribution to each PC axis and trait names labeled in orange at the arrow tips. The percentage of variance explained by each axis is indicated on the corresponding axis labels. Top and right plot margins contain warped landmark grids visualizing the minimum (left/bottom) and maximum (right/top) shape deformation along each PC.

Eastern Pacific Ocean (Supplementary Figure S6). Anagenetic dispersal events were estimated the most frequent in explaining the biogeography of the Pomacentridae family, followed by narrow sympatry (or parapatry). Estimates of founder, vicariance and nested sympatry events were relatively low, with an average of 30, 30 and 70 counted events, respectively, over the 50 stochastic reconstructions (Supplementary Figure S7).

Phylogenetic comparative analysis of trait evolution

Regime shifts and convergence in colour and morphology

Using the phylogenetically corrected Bayesian Information Criterion (pBIC), `ℓ1ou` analyses detected at least one independent adaptive regime shift along every PC axis examined for both colouration and morphology. Across all univariate trait axes (colour and morpho PC1–8), up to seven shifts were inferred on a single axis, revealing extensive heterogeneity in the selective regimes shaping damselfish phenotypes (Figure 5).

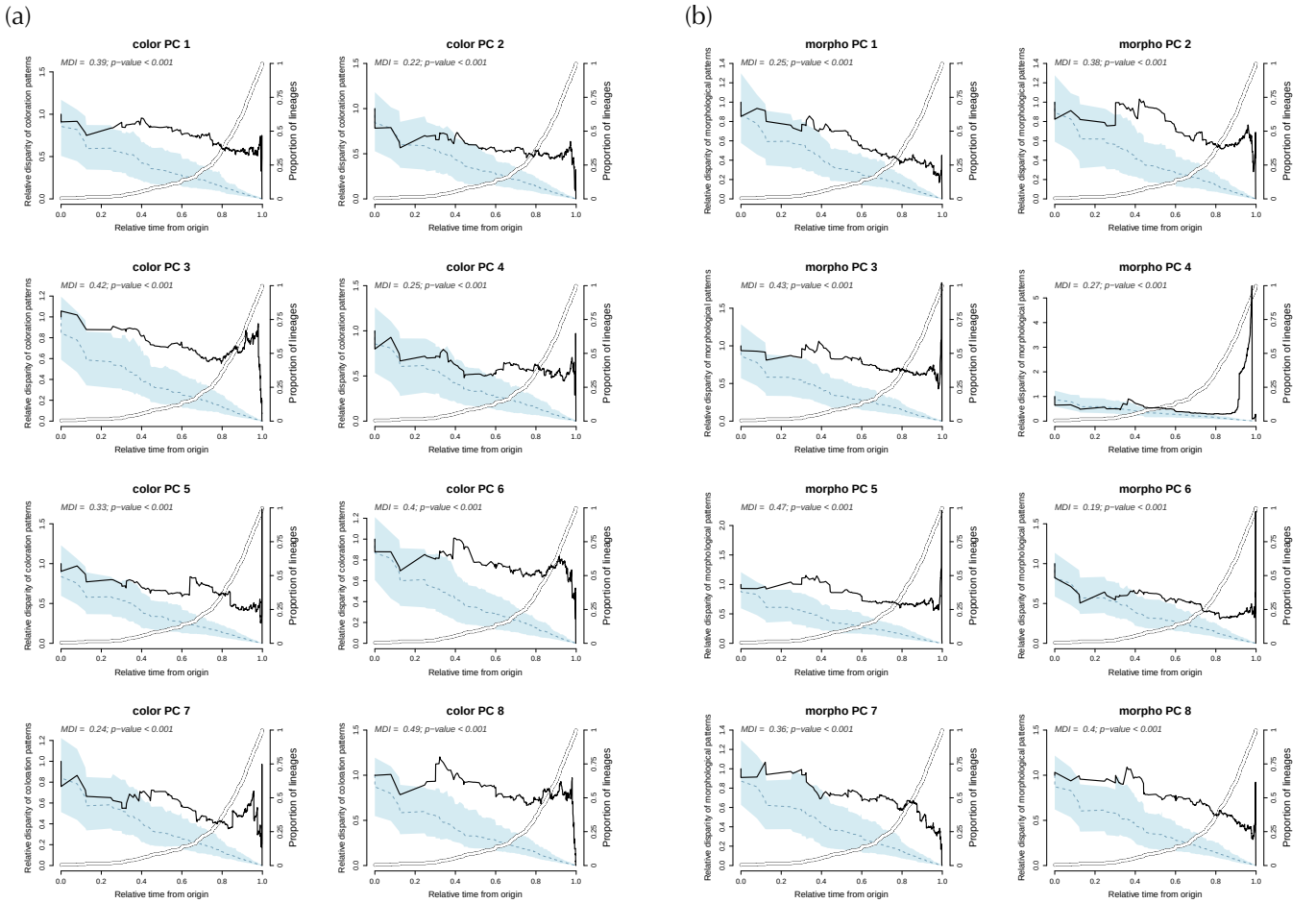

Fig. 4: Disparity-through-time (DTT) plots for color (a) and morphological (b) traits across principal component axes 1–8. Each facet displays the observed disparity pattern (black line), the lineage-through-time (LTT) curve (thick line) for interpretability, and the expected disparity under a Brownian Motion model (dashed blue line with blue shading representing the 95% confidence interval). The morphological disparity index (MDI) and associated p-value are reported at the top of each panel to quantify deviation from BM expectations.

Convergent shifts in colour traits.

Convergent evolution—defined here as independent lineages evolving toward the same selective regime—was detected in colour PC1, PC2, and PC6. In colour PC1, two major convergent regimes were identified: one uniting a subclade of *Pomacentrus* with a subclade of *Chrysiptera*, both evolving toward darker colouration; and a second regime linking the entire *Amphiprion* clade with a nested lineage within *Chrysiptera*, converging on a contrasting region of phenotypic space. These patterns highlight repeated, directional evolution along shared trait axes. Additional, more localized shifts were identified on terminal branches, indicating recent, lineage-specific divergence. In colour PC2, convergence was detected between *Neoglyphidodon* and *Amphiprion* species toward positive values along this axis, while a broader regime shift at the base of the family—including early-diverging genera, such as *Stegastes*, *Lepidozygus*, *Plectroglyphidodon*, and *Parma*, reflects a shared evolutionary trend toward reddish hues. Similarly, in colour PC6, convergent evolution was observed between *Amphiprion* and a subclade of *Pomacentrus*, both evolving similar antero-dorsal colour patterns differentiating from the baseline pattern. Additional shifts were detected in shallow clades or terminal lineages, including those within *Dascyllus* (PC3, PC5), the *Amphiprion* clade (PC4, PC8), and again in *Pomacentrus* (PC6), suggesting finer-scale diversification in colour pattern evolution.

Convergent shifts in morphological traits.

Signatures of morphological convergence were also recovered, particularly in morpho PC1, PC3, and PC7. In morpho PC1, convergence toward elongate body shapes was identified between distantly related taxa, including the genera *Neopomacentrus*, *Teixeirichthys*, *Pristotis*, and multiple species of *Chromis* and *Azurina*. Morpho PC3 captured convergence between the *Pomacentrus*-*Amblypomacentrus* clade and the *Pycnocromis* lineage, characterized by similar ratios of snout length, pelvic fin, and peduncle area. A second convergence in this axis linked *Abudefduf* and *Dischistodus*, which share similar head and eye proportions. In morpho PC7, we identified two major convergence regimes. The first included the

clade encompassing *Amphiprion*, *Neopomacentrus*, *Teixeirichthys*, and *Pristotis* species with a distant clade including *Pycnocromis*, *Dascyllus*, and *Azurina* species. The second convergent regime united the entire *Amblyglyphidodon* clade with a subclade of *Chrysiptera*. Both independent convergent regimes evolved towards the same morphological space in body length and caudal peduncle proportions. Unlike colour traits, shifts in morphological axes often encompassed deep-diverging clades, with additional regime shifts inferred in morpho PC2, PC4, PC5, PC6, and PC8, reflecting repeated and phylogenetically widespread episodes of morphological innovation.

Pagel's λ model was selected from multivariate GLS analysis on the eight colour PCs against each one of the three ecological traits analyzed in this study (diet ecotype, habitat type, and symbiosis), indicated by the lowest EIC value (Supplementary Table S1). Phylogenetic MANOVAs showed a significant effect of habitat type and the type of symbiosis relationship on colour patterns (Pillai: $S = 0.153, p = 0.009$ and Pillai: $S = 0.115, p = 0.003$, respectively; Supplementary Table S2). In contrast, colour patterns were found to be phylogenetically independent from diet ecotype (Pillai: $S = 0.063, p = 0.0117$).

Similar to colour patterns PCA multivariate GLS analysis, Pagel's λ model was selected based on the lowest EIC value as the evolutionary model to better explain the relationship between the seven morphological PCs and each ecological trait (Supplementary Table S3). The distinct classes within diet ecotype and symbiosis were found to exert a significant effect in the evolution of morphological patterns (Pillai: $S = 0.103, p = 0.002$ and Pillai: $S = 0.069, p = 0.046$, respectively; Supplementary Table S4). Morphological traits showed to be phylogenetically independent from habitat type (Pillai: $S = 0.091, p = 0.274$).

Univariate likelihoods of the distinct evolutionary models (BM, WN, OU and OUM) indicated that the colour traits, represented by each colour PC, in the Pomacentridae family evolve in response to one or several optima (Supplementary Table S5), following an Ornstein-Ulhenbeck model [50]. The model comparison revealed that different colour PCs, such as col PC1 and col PC3 for diet ecotype, col PC4 and col PC8 for habitat type, and col PC3 and col PC4 for symbiosis, show distinct evolutionary optima based on their ecological traits.

Univariate analyses on each of the morphological PCs revealed that morphology evolution followed either a Brownian Motion or an Ornstein-Uhlenbeck model with a single optimum, indicating either unconstrained stochastic evolution or stabilizing selection toward a specific optimum (Supplementary Table S6). These findings indicate no evidence of evolutionary divergence in morphological traits linked to the observed variation in any of the ecological traits.

Discussion

Our findings reveal a modular architecture of trait evolution in Pomacentridae. While body shape evolution shows bounded divergence consistent with selection toward ecotype-specific adaptive peaks, colouration exhibits greater ecological lability, evolving with early bursts and widespread convergence across distant lineages. Morphological and colour traits show partial integration, but respond differently to ecological regimes: pelagic, demersal, and reef-associated taxa display distinct colour-morphospace trajectories. Notably, clownfish colour convergence does not represent a unique trajectory, but rather echoes deeper evolutionary processes found throughout the family. By mapping the evolution of ecological, morphological, and colour diversity onto a shared phylogenetic and biogeographic framework, our study highlights how ecological specialization drives repeated innovation in reef fish phenotypes—and how colour, long considered a species-level quirk, may follow macroevolutionary patterns as predictable as form and function.

The present study aims at exploring the potential for evolutionary convergence in the Pomacentridae family by examining the relationship between colouration, morphology and ecological factors such as habitat, diet and symbiotic relationships. Specifically, we hypothesized that damselfishes inhabiting similar reef environments or exploiting similar food resources would exhibit convergent patterns of colouration and morphology, even if they were not closely related phylogenetically. To test this hypothesis, we conducted a comprehensive analysis of colouration patterns and morphological traits across 342 pomacentrid (314 damselfish and 28 clownfish) species, representing a range of symbiotic relationships, habitat preferences or types, and dietary specializations. We applied comparative phylogenetic methods to identify patterns of convergence. By investigating the evolutionary mechanisms underlying colouration pattern in damselfishes, our study represents the first attempt to explain the diversity of colouration patterns exhibited by this family of reef fishes from an evolutionary perspective.

Ancestral state reconstructions

Ancestral state reconstructions unveils the evolutionary history of dietary, habitat and symbotic preferences of ancestral Pomacentriae species. These analysis indicate an intermediate omnivory diet and farming behavior in non-reef environments of the common ancestor, with subsequent transitions to diverse ecological niches across the phylogeny. Ancestral reconstruction of diet ecotype aligns with that of [34], where they showed that asymmetry in dietary strategies constituted an important driver of damselfish diversification.

Considering the origin of coral reef fishes is estimated to be around 55 million years ago [51–53], a similar time frame for the emergence of the clade, the estimated 'non-reef' habitat preference of the pomacentrids' common ancestor results in a reasonable inference. Ancestral state reconstruction points to a predominantly "non-ree"

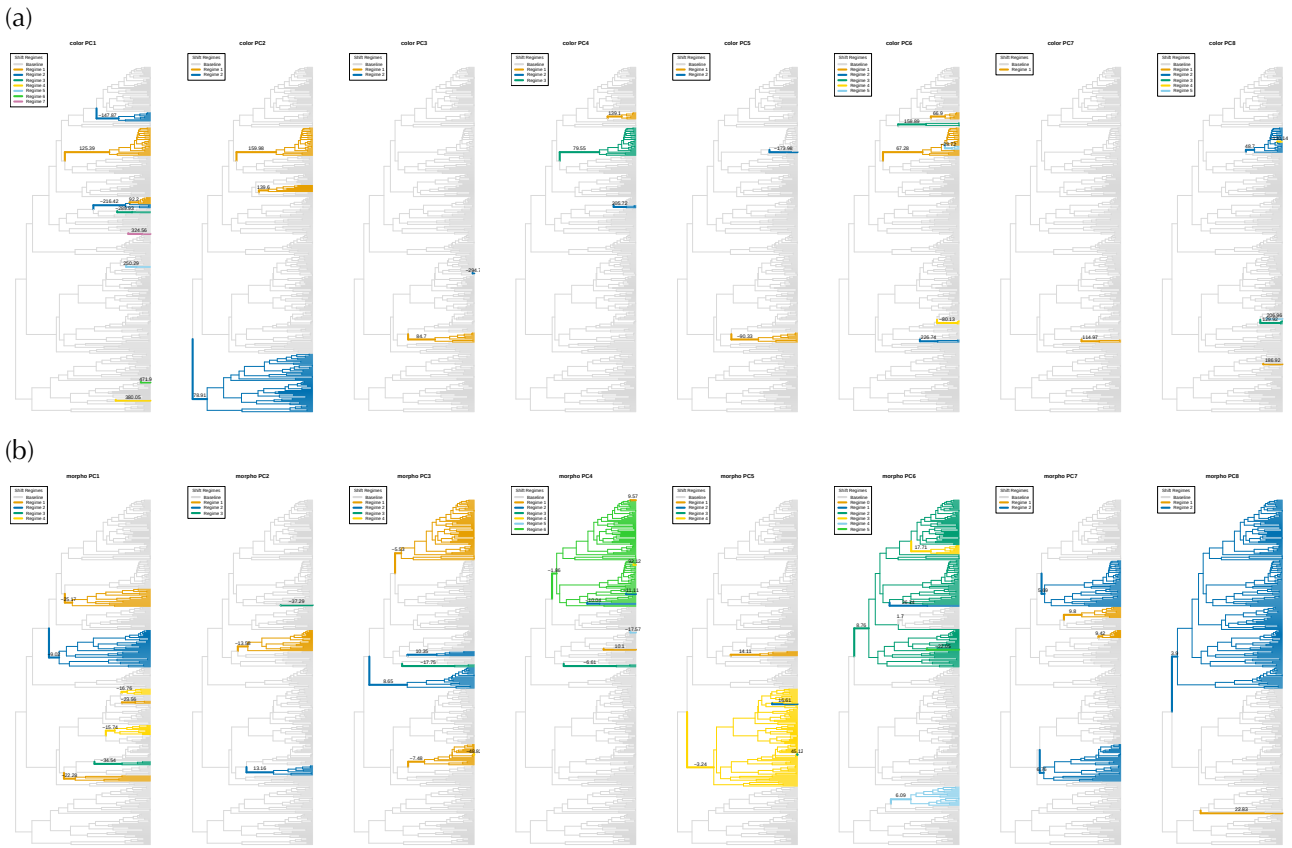

Fig. 5: L1OU-inferred adaptive regime shifts across the damselfish phylogeny based on univariate analyses of principal component axes. Panel (a) shows regime shifts for colouration traits (PC1–PC8), while panel (b) displays shifts for morphological traits (PC1–PC8). Grey branches indicate the baseline (ancestral) regime, and coloured branches represent inferred shifts to alternative selective regimes. Identical colours across phylogenetically distant clades indicate convergent evolution toward similar trait optima. Convergent shifts were detected in colouration PCs 1, 2, and 6, and in morphological PCs 1, 3, 4, and 7. The estimated shift value from L1OU is indicated at the start of each regime shift. Each panel includes a regime legend in the top-left corner, summarizing the number and identity of inferred regimes. Shifts were detected based on the most conservative criterion 'pBIC'.

habitat preference in the earliest pomacentrids. This suggests that initial diversification in the group may have exploited alternative marine habitats such as mangroves, rocky shores, seagrass beds, or even soft-bottom substrates prior to widespread reef occupation. Our estimates show that initial movement of pomacentrids into reef environments likely occurred between 40 and 50 million years ago, corresponding to an epoch when reef complexity and coral diversity were increasing, presenting new ecological opportunities [54]. The emergence of the pomacentrid clade is nested within this broader wave of diversification, suggesting that damselfishes evolved contemporaneously with the flourishing of reef systems, but not necessarily in direct association with reefs at their origin. This dynamic aligns with patterns seen in other prominent reef fish families [51, 55, 56] and underscores the importance of ecological opportunity in driving habitat transitions.

Our results indicate a higher probability for the common ancestor of all pomacentrids to exhibit commensalistic behavior rather than having no symbiotic relationships. However, this inference appears improbable since the 'free-living - obligate symbiont' continuum exhibits a strong asymmetry, favoring the development of symbiotic relationships [57–59]. Analogous to the habitat reconstruction, refining the model to account for this pronounced asymmetry could enhance the accuracy of the inferences.

Shifts towards symbiotic relationships, particularly to mutualism, are observed at key nodes in the evolutionary tree. Transition from no symbiotic relationship to mutualism occurrs three times. The most ancient transition gives rise to the clownfish clade and its mutualistic relationship with sea anemones, aligning with previous studies [11, 60], highlighting the adaptive radiation triggered by this ecological specialization. The other two transitions correspond to recent adaptations to live in association among sea anemones in the sister species *Amblypomacentrus breviceps* and *A. claurus*, and *Dascyllus trimaculatus* and *D. abisella*. While these species do not exhibit a lifelong dependency on their association with sea anemones, they have been shown to rely to some extent on the protective shelter provided by these organisms, which can impact their life histories [61–63].

Ancestral biogeographic reconstruction indicate that the ancestral Pomacentridae had a widespread distribution

in the Tethys Sea and the Pacific Ocean, with anagenetic dispersal events being the predominant mode of biogeographic evolution. This pattern aligns with the findings of [51] and [64]. The widespread distribution of the ancestral Pomacentridae is likely linked to the geological evolution of the Tethys Sea, a vast ocean that existed between the ancient supercontinents of Gondwana and Laurasia during the Mesozoic era. As the Tethys Sea gradually closed due to the northward movement of the African and Indian plates, it led to the formation of the Mediterranean Sea and the Indo-Pacific region. This geological event, along with the expansion and contraction of coral reef habitats, played a crucial role in shaping the biogeographic patterns observed in damselfish and other coral reef organisms [55]. The predominance of anagenetic dispersal events, where lineages gradually expand their ranges over time, suggests that the Pomacentridae were able to take advantage of the expanding coral reef habitats in the Indo-Pacific region as the Tethys Sea closed. This is supported by the fossil evidence from the Eocene deposits in Monte Bolca, Italy, which indicate that many modern reef fish families, including the Pomacentridae, were already present in the Tethys Sea during this time [64]. Other inferred biogeographic processes, such as narrow sympatry, subset sympatry, vicariance, and founder events, likely reflect the more recent responses of damselfish lineages to the contraction and fragmentation of coral reef habitats, as well as the formation of major marine biogeographic barriers like the Isthmus of Panama [51]. These events may have led to the diversification and speciation of damselfish within the Indo-Pacific region, contributing to the high biodiversity observed in this group today.

Evolution of colouration patterns

The PCA on Procrustes-transformed images reveals distinct patterns in colouration. PC1 primarily differentiated between light-coloured and dark-coloured fish, being detected by `ℓ1ou` as a trait showing evolutionary diverging early on in the evolution of the Pomacentridae family. Specificallyl, this colour trait divergence is associated with the first speciation event occurring at the root of the tree, approximately 55 Million years ago. The shift observed in this monophyletic clade, inlucing the genera *Stegastes*, *Lepidozygus*, *Plectroglyphidodon*, *Siliparma*, *Hypsipops*, *Nexilosus*, *Microspathodon*, *Parma*, and *Mecaenichthys*, exhibit certain common features. They are in their majority herbivorous damselfishes that feed on algae, most of which are 'farmers', tending 'gardens' of filamentous algeae [40]. Only the species *Lepidozygus tapeinosoma* and the two *Siliparma* species exhibit a planktivory and omnivory diets, respectively. Additionally, most of these species are predominanly occuring in rocky reefs. These species appear to be darker than the average pomacentrid, characteristic that could be associated to benthic herbivory and rocky reef adapations. Indeed, univariate models indicated a Ornstein-Ulhenbeck mode of this colour trait evolution in association with dietary strategies, habitat preferences and symbiotic behaviors.

PC2 distinguishes rostral white bars and orange body colouration from those lacking these features. These features constitute the most characteristic pattern of the clownfish clade compared to the rest of the family. Indeed, `ℓ1ou` detects an independent shift at the root of the clownfish clade, suggesting that the rostral white bar and orange colouration was potentially associated with the origination of the clade. Vertical bars patterning have followed a caudal to rostral loss in the evolution of clownfishes, with the common ancestor exhibiting a three-bar patterning [65]. Importantly, whereas clownfish species show a ontogenetic gradual loss of vertical bars in a caudal-rostral manner until reaching the species phenotype [65], the rostral bar is the most persistent feature in all clownfishes. Our result adds up to the growing literature on the evolution of colouration in clownfishes [see 11], emphasizing the importance of the rostral vertical bar as a precursor pattern in the clade and potentially its implication in the mutualistic association with sea anemones.

Evolutionary convergence on PC2 colour pattern is detected on four distant related species (*P. pavo*, *C. cyanea*, *C. taupou* and *A. cyanea*), all four exhibiting strikingly similar electric blue body colouration. However, we do not find support on any ecological trait to be associated with divergent patterns in colour PC2. Instead, univariate analyses support a single-optima Ornstein-Ulhenbeck model of evolution for all three ecological traits (diet, habitat and symbiotic behavior). These four species exhibit a variety of omnivorous and planktivorous feeding behaviors, with not known symbiotic associations and inhabit from rocky to sandy areas of lagoon reefs. Three species occur in sympatry (*P. pavo*, *C. cyanea*, and *C. taupou*) in the Indo-Pacific, while *A. cyanea* is found in the Atlantic Ocean. Although we do not find support of any ecological trait in association with the convergent phenotypes, we cannot disregard the possibility of other factors that could potentially be attributable to this convergence. Traits related to courtship during mating or hunting strategies (not evaluated in this study) could potentially have contributed to this convergence. Strong sexual selection in colouration could drive particular phenotypes [66–68], or hunting efficiency in predator-prey interactions could exert selective pressures towards similar phenotypes [69, 70].

Similarly, evolutionary convergence in towards and away from vertical bar patterning, represented by colour PC6 and PC7, was found across fat-related taxa but none of the ecological traits analyzed explains this pattern of convergence, indicating the need for further investigation into other potential factors that may influence the evolution of vertical bar patterning in these taxa. Ornstein-Ulhenbeck with multiple optima was the best model explaining the evolution of the contrasting patterns within the central and caudal body areas, represented by colour PC8, associated to different habitat types. Contrasting patterns are often attributed to a camouflage strategy in reef fishes [71], and similar patterns may have evolved to match the visual characteristics of their specific reef habitats [47].

The univariate analyses support the Ornstein-Ulhenbeck model with multiple optima for the evolution of light versus dark (PC1 and PC4), vertical bicolour

(PC3), and contrasting (PC8) colouration patterns. Specifically, colour PC3 exhibits convergence towards different optima based on diet ecotype and symbiotic relationship, while colour PC4 shows similar dynamics with habitat type and symbiotic relationship. These findings highlight the correlative nature of various ecological traits exerting similar pressures, emphasizing the complexity inherent in integrating them into macroevolutionary analyses [72]. Additionally, multivariate GLS analyses further elucidates the relationship between colour patterns and ecological traits, revealing significant associations with habitat type and symbiotic relationships. This underscores the ecological influences on colouration evolution, although colour patterns were found to be phylogenetically independent from diet ecotypes, suggesting that dietary preferences may not be a major driver of colouration evolution in this family.

Morphological evolution

We find multiple evolutionary shifts in the rate of evolution of morphological traits with signatures of convergence. The most notable examples are found in PC2, strongly representing the maximum body size (total length), in which we found three signatures of convergence, including large and far-related clades. A large group of distant clades, conforming the *Pomacentrus*, *Amblypomacentrus*, *Neopomacentrus*, *Pycnochromis*, and *Dascyllus* genera, exhibited evolutionary convergence towards small body sizes. Conversely, *Parma* and *Abudefduf* genera manifest evolutionary convergence towards larger body sizes. Similar trend but with a different signature of convergence is observed in *Chromis punctipinnis*, *M. bairdii*, *M. dorsalis*, *N. latifrons*, and *H. rubicundus* species. Body size evolution has been linked to the trophic group and feeding behaviors of reef fishes [53]. However, we do not find support to this association, with a Brownian-Motion fashion the most likely explanation to the body size evolution in pomacentrids. Our result also aligns with findings in [34], where neither body size nor body depth was found associated with dietary strategies. A remarkable example of evolutionary shift was found in the *Chrysiptera* clade, encompassing *Chrysiptera*, *Pomachomis*, and *Dischistodus*. This group exhibited a evolutionary shift towards small body sizes, and the origin of the *Dischistodus* clade suffered a new shift towards larger morphotypes. These dynamics reflect the evolutionary lability of the Pomacentridae family to shift between extreme morphotypes.

Convergent evolution in the Pomacentridae family

Our study reveals convergent evolution in both colouration and morphology across distantly related taxa. Convergent colour patterns found to be driven by similar habitat types, are suggested to serve as camouflage or signaling functions [46, 47]. In damselfishes, the darker colouration in the three freshwater species may serve a mimicry function, enhancing camouflage by better matching the typically shaded, tannin-rich, or murky conditions of freshwater environments. A similar pattern is observed in symbiotic species, with commensalists, corresponding to algae-farming species, exhibit darker colouration, potentially to mimic the green and brown hues of their algae gardens. In contrast to commensalists and freshwater specialists, mutualists like clownfishes, which are inhabiting sea anemones, exhibit lighter colourations towards the orange hue and white vertical bars. These opposite patterns to benthic species may serve as a mechanism for species recognition, as camouflage and protection are provided by the sea anemone they inhabit [73]. Strong competition for habitat may exacerbate the importance of differentiation between individuals and prevent the invasion of resembling species [74], thus providing a possible explanation for the distinct vertical bar patterning observed in coexisting clownfish species [75, 76].

Damselfishes with specialized feeding strategies, such as algae grazers versus plankton feeders, exhibit convergent morphological adaptations in head and peduncle ratio. Additionally, commensalistic and mutualistic species display specific adaptations in head, eye, and snout ratio. These results align with [4], highlighting morphological convergence towards similar trophic strategies, albeit contrasting with the findings of [34], who reported no associations between body size, depth, farming, and dietary strategies. It is important to note the methodological differences between studies, with [34] using body size and depth metrics, while [4] and the present study employed landmark-based geometric morphometric methods.

Conclusion

The biology, ecology, and evolutionary history of the Pomacentridae family highlight their importance as a diverse and adaptable group of coral reef fishes [77]. These fish exhibit a remarkable diversity of colour patterns and morphological adaptations that appear to be closely linked to their ecological roles and habitat preferences. By continuing to explore the factors driving their remarkable diversity, such as the potential for evolutionary convergence in colouration and morphology, researchers can gain valuable insights into the processes that have shaped the remarkable biodiversity of pomacentrids in temperate and tropical marine habitats. This research contributes to a growing body of evidence suggesting that damselfishes are a model system for studying the interplay between form, function, and ecology in the evolution of marine organisms. By leveraging the wealth of information available on this charismatic group of fishes, we uncovered new insights into the processes that have shaped the remarkable diversity and adaptability of life in the ocean.

Our study unraveled the intricate evolutionary relationships between colour patterns, morphological traits, and ecological preferences in the Pomacentridae family. We found evidence of convergent evolution in both colouration and morphology across distantly related taxa, driven by similar habitat types and ecological pressures. Ancestral state reconstructions revealed asymmetric transitions towards diverse ecological niches, representing different dietary, habitat, and symbiotic preferences. Our study

brings insights into the interconnected nature of various ecological traits exerting similar evolutionary pressures. This research contributes to a growing body of evidence suggesting that damselfishes are a model system for studying the interplay between form, function, and ecology in marine organisms. Future research elucidating the underlying mechanisms driving convergent evolution in colouration and morphology, while considering ecological traits related to mating behavior or predator-prey interactions, could further expand our knowledge on the adaptive dynamics of these traits. Overall, our study represents a significant step towards unraveling the evolutionary patterns and processes shaping the diversity of colouration and morphology in the Pomacentridae family.

Methods

Coloration and morphological datasets

We collected 1,183 pictures from 342 species of pomacentrids from different reef fish image databases (see details in Supplementary Material), selecting only images showing the fish's full body in a lateral view. From each of the species inventory of images collected, we selected the best quality and most representative image of the species colouration pattern, excluding juvenile images. We applied minimal image post-processing for visual consistency. Underwater colour correction was performed using a custom Python script, which applied contrast-limited adaptive histogram equalization (CLAHE) in LAB colour space, adaptive histogram equalization for white balancing, saturation enhancement, and denoising using OpenCV [78] and scikit-image [79] libraries. Finally, we set 46 morphological landmarks (Figure S1) on each image using `FIJI` software [80]. Using the landmarks dataset, we carried out Procrustes transformation, superimposing and align each image to a 'mean shape' using the *geomorph* R package [81]. After the transformation, we applied a custom script to handle missing values (NAs) generated during the alignment process. This script interpolated NAs using surrounding pixel information to ensure smooth and complete images.

Colouration patterns were quantified using Principal Component Analysis (PCA) applied to a dataset of 1,183 standardized RGB images of individual specimens, processed through a custom pipeline developed for this study. Prior to PCA, all images were standardized for resolution, spatial extent, and alignment to ensure comparability across images. Each image was then linearized by flattening the red, green, and blue (RGB) pixel channels into a single vector, resulting in a matrix with individual specimens as rows and concatenated RGB pixel values as columns. PCA was performed on this matrix, and the first eight principal components —explaining 50.5% of the total variance— were retained for downstream analyses. To reduce the influence of individual-level noise and enhance species-level patterns, individual PCA scores were averaged at the species level. While averaging images by species reduces resolution at the intraspecific level, it emphasizes dominant species-specific colouration patterns —the primary focus of this study. This approach was also necessary given the species-level phylogeny and the lack of correlative information linking individual specimens to phylogenetic relationships, making species-level aggregation more appropriate for downstream comparative analyses.

We applied Procrustes superimposition to the landmarks dataset to extract morphometric traits from homologous landmarks. To focus on species-level morphological patterns and minimize the influence of individual-level variation or potential landmark noise, we averaged individual morphometric traits at the species level. This approach allowed us to retain the primary species-specific morphological features while reducing confounding intraspecific variability. From these averaged values, we derived multiple ratios reflecting ecologically relevant features associated with visual acuity, swimming performance, and vertical positioning in the water column, including body ratio, head ratio, snout ratio, eye-height ratio, peduncle ratio, and snout angle [82, 83]. Given the phylogenetic conservation of morphometric traits, we applied phylogenetic PCA on 11 morphometric traits using the `phyl.pca` function from the *phytools* package [84] to account for the evolutionary relationships between species. This approach corrects for shared evolutionary history and isolates species-level morphological patterns. The morphometric dataset was subsequently constructed using eight PC axes of the phylogenetic PCA, accounting for 92.4% of the total morphometric variation.

Time calibrated phylogeny

We utilized the latest well-resolved time-calibrated phylogeny provided by [34], encompassing 345 out of the 422 extant pomacentrid species. Subsequently, the phylogenetic tree was pruned using the *ape* R package [85], retaining 342 species for which we had comprehensive colouration, morphological, and ecological data (1).

Ecological data

We utilized dietary ecotype, with 'benthic' herbivory, 'pelagic' planktivory, and 'intermediate' omnivory classes, and farming ('yes'|'no') information from [34]. Furthermore, ecological data pertaining to habitat type obtained from `Fishbase` using the *rFishBase* R package [86]. Habitat type refers to the primary or most common habitat of the fish, extracted from the comments section of the *Fishbase* entry and corroborated with additional literature and sources. To simplify trait reconstruction analysis due to the diverse habitats of damselfishes, habitat type was categorized into five classes. The 'sea anemones' class includes species closely associated with or inhabiting sea anemones, represented by the clownfish clade and two Amblypomacentrus (*A. breviceps* and *A. clarus*) and two Dascyllus (*D. albisella* and *D. trimaculatus*) species. The 'freshwater' class comprises species capable of inhabiting freshwater environments like *Neopomacentrus aquadulcis*, *N. taeniurus*, and *Stegastes otophorus*. The 'non-reef' class encompasses species that inhabit seagrass beds, rubble, or sandy habitats, while the 'rocky reef' and 'coral reef' classes represent species associated with these specific reef environments. Additionally, a three-class categorization was established based on the type of symbiotic association: 'mutualistic' (e.g., clownfish and sea anemones), 'commensalistic' (e.g., farming damselfishes [87]), or 'free-living' species with no direct association with another species.

Ancestral state reconstructions

We reconstructed ancestral states (ASR) of discrete traits from the ecological dataset, including diet, habitat type, and symbiosis. ASRs were performed using the `fitMk` function from the *phytools* R package [84] and the `corHMM` function from *corHMM* R package [88]. We tested transition matrices with all equal rates (ER), symmetric rates between states (SYM), all rates different (ARD), and up to three directional rates (DIR1-3) with and without hidden states. We compared and selected the best model based on the AIC criterion [89] to infer joint and marginal probabilities of ancestral states using `corHMM`. Finally, 100 stochastic maps using the selected transition matrix were generated with `make.simmap` *phytools* function. A weighted consensus trait reconstruction was then estimated by combining the mapped edge lengths across all 100 stochastic maps, where each map was weighted according to its likelihood. ASR plots were produced using *phytools* R package.

Ancestral biogeographic ranges

Ancestral biogeographic reconstructions of the Pomacentridae family were carried out using the R package *BioGeoBEARS* [90]. We classified species current distribution into five bioregions, being Indian Ocean (IO), Indo-Australian Archipelago (IAA), Central Pacific (CPO), East Pacific (EPO) and Atlantic Ocean (AO). We chose to perform a stratified analysis in which beogeographic reconstruction was divided and conducted in five time intervals corresponding the the geological epochs Eocene (56-34 Mya), Oligocene (34-23 Mya), Miocene (23-5 Mya), Pliocene (5-3 Ma) and Pleistocene-Holocene (3-0 Mya). A sixth region, Tethys Sea (TS) was allowed from the Eocene to the Oligocene, and then deactivated following the geological closure and disappearance of the Tethys sea. Similarly, the IAA region was only activated during the Pliocene and Pleistocene-Holocene stratus, as before the Pliocene the IO and the CPO were widely connected. For each time interval, we created a distance matrix containing distances among bioregions. Distances were calculated using geological coral reef habitat maps of each time period from [91] and calculating pariwise geodesic distance between coral suitable areas of each bioregion using the function `geoDist` from the *oce* R package [92]. We conducted ancestral biogeographic reconstruction for the Pomacentridae family employing maximum likelihood estimation to determine parameters for DEC, DEC+J, DIVA, DIVA+J, BAYAREA, and BAYAREA+J

models. We selected of the optimal model based on AIC and LRT criteria. Subsequently, we employed Biogeographic Stochastic Mapping (BSM) with the chosen model, generating 100 stochastic maps illustrating the co-occurrence between lineages across the phylogeny.

Partitioning of phenotypic variation and phylogenetic signal

To evaluate the extent to which species identity and ecological factors explained phenotypic structure, we conducted permutational multivariate analyses of variance (PERMANOVA). These analyses were performed separately for morphological and colour trait spaces of the individual-level datasets, first using species identity as a grouping factor, and subsequently using ecological traits (diet, habitat, and symbiosis) as predictors of phenotypic composition across principal component axes. Phylogenetic signal in morphological and colour traits was estimated aligned images and Procrustes landmark coordinates, respectively, using multivariate extensions of Blomberg's K with the function `physignal` from *geomorph* R package [81]. For colour traits, images were aggregated by species to compute species-specific mean values for each pixel across the RGB channels, which were then reshaped into a 3D array with dimensions [pixels, RGB channels, species]. For morphological traits, individual geometric morphometric coordinates were aggregated by species to match the phylogenetic tree. We also carried out Mantel tests comparing phylogenetic distances with species-averaged colour PCA distances and with species-averaged morphological trait distances, using the `mantel` function from *vegan* [93]. Further, to characterize the temporal dynamics of trait disparity, we implemented disparity-through-time (DTT) analyses on the principal component scores of both morphological and colour data using `dtt` function from *geiger* [94]. These analyses estimate relative phenotypic dispersion through evolutionary time, allowing inference of clade-wide patterns of convergence or divergence. Significance was assessed via 100 Monte Carlo simulations, and morphological disparity index (MDI) values were computed to evaluate deviation from Brownian expectations.

Detection of trait shifts and convergent evolution

To explore potential convergence towards phenotypic optima without predefined selective regimes, we employed the `ℓ1ou` package [95]. This model-based approach utilizes a regularized least absolute shrinkage and selection operator (LASSO) method to determine the optimal number of selective regimes within a phylogeny, accommodating single or multiple traits. By applying different Ornstein-Uhlenbeck (OU) models to the phylogeny, `ℓ1ou` aims to discern the requisite number of selective regimes for describing trait evolution, often collapsing similar regimes together. Convergence, as detected by `ℓ1ou`, manifests through either identical (collapsed) or highly similar sets of OU parameters across distantly related taxa. This approach benefits from its ability to discern emergent patterns from the empirical data, avoiding predefined categorical classifications. We assessed convergence patterns independently on morphological and colouration PC datasets, analyzing both the full set of combined PCs and each PC axis separately within each dataset. We utilized the Bayesian information criterion adjusted for phylogenetic dependencies (pBIC) for determining the number of shifts in the `ℓ1ou` model and assessing the robustness of the detected convergent regimes [95]. We performed 100 bootstrap replicates of the `ℓ1ou` analysis to evaluate support for the detected convergence regimes. By rerunning the method on resampled datasets, we quantified how consistently these regimes were recovered across iterations. Convergence patterns with high bootstrap frequency (e.g., recurring in $\geq$ 90% of replicates) were considered strongly supported.

Ecological associations of trait evolution

To examine the influence of multiple traits on morphology and colouration patterns, we used a multivariate phylogenetic analysis of variance (*p*MANOVA) [96, 97]. We first fitted linear models using penalized likelihood on generalized least square models (GLS) considering several alternative evolutionary models (BM, OU, EB, or Pagel's λ) for the first eight axes of the colouration PCA and the first eight axes of morphometric traits PCA, separately. We compared the extended information criterion (EIC) from the different evolutionary models with 100 bootstrap iterations [98]. We then conducted a *p*MANOVA on the best GLS model fit using the *Pillai* test.

Further, we conducted Maximum Likelihood (ML) estimation of colouration and morphology the evolutionary rates. We analyzed the colour patterns and morphology datasets separately, employing a multivariate approach with the R package *mvMORPH* [99]. This method was used to determine if colouration patterns and morphology are phylogenetically conserved within clades.

To test whether ecological regimes were associated with shifts in trait values while accounting for phylogeny, we employed both phylogenetic ANOVA and phylogenetic generalized least squares (PGLS). Phylogenetic ANOVA was used to test differences in individual traits across levels of each ecological variable. In parallel, PGLS models were fitted for each trait, with ecological traits included as predictors and species-level trait means as responses. These models used a variance–covariance matrix derived from the phylogeny and evaluated significance via likelihood-based hypothesis testing.

As a second part of these analysis, we employed a univariate approach for each dimension of colouration and morphology, using models that account for density effects or ecological traits. We compared univariate OU, BM, and WN models fitted with the R package *geiger* [94] to an OU model with multiple optima determined by a discrete trait (OUM) fitted with the R package *OUwie* [100]. We conducted all phylogenetic analyses using R [v.4.3.2; 101], utilizing functions from both the *phytools* package [84] and the *ape* package [85] for data manipulations.

Supplementary information

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Declarations

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Conflict of Interest Statement

The authors declare no competing interests in the publication of this work.

Data and code availability

Scripts with instructions to reproduce the analysis and figures can be freely accessed at https://github.com/agarciaEE/DamselfishTraitEvolution2025. Access to these full data set will be provided upon acceptance of the manuscript.

Author contribution

AGJ, TG and NS designed the study. AGJ conducted the research and analyses and wrote the manuscript. TG and NS contributed to the interpretation of the analyses, and the writing of the manuscript. All authors participated in reviewing, correcting, and approving the final version of the manuscript.

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