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Original Article

Modularity underlies the morphological evolution of Appalachian salamanders

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Abstract

Modularity is a pattern whereby traits evolve as inter-related systems, resulting in lower covariation between modules than within. Modularity may facilitate diversification by permitting the phenotype to independently respond to selection. The Appalachian Mountains are a biodiversity hotspot for lungless salamanders (Plethodontidae). Despite their ecological diversity, putative catalysts of their radiation in the Appalachians are poorly understood. We test whether (i) Appalachian salamanders exhibit modularity in functional, morphological characters and (ii) if the rate of evolution—a fundamental macroevolutionary signal of ecological opportunity—varies among modules and if their rates are correlated. We find that Appalachian salamanders exhibit modularity under two different models in which their head, trunk, limbs, and tail or the head, body, and limbs act as separate modules. The three major clades, *Plethodon*, *Eurycea*, and *Desmognathus*, vary in the presence and extent of modularity. Across Appalachian salamanders, modules exhibit nonsignificant differences in rates of evolution (~1.5-fold), with the trunk and tail modules exhibiting the slowest and fastest rates, respectively. We also find that the rate of modular evolution can be highly correlated, suggesting that modules may respond similarly to ecological opportunity. These results suggest that modularity underlies the morphological diversity of Appalachian salamanders by permitting localized regions of the phenotype to respond independently to selection, but not evolve at different rates.

Keywords: continental radiation, integration, macroevolution, functional morphology, diversification, microhabitat

Introduction

The sheer diversity of complex life inspires hypotheses about its many catalysts. Extrinsic drivers such as climatic or environmental change alter the adaptive landscape and spur novel evolutionary trajectories for lineages—extinction for some, competitive release for others (Jablonski, 2001; Ghezeyalagh et al., 2022; Meredith et al., 2011). Intrinsic drivers such as functional innovations may fundamentally change how an organism interacts with the environment, providing access to previously unattainable resources such as wings enabling powered flight (Heard & Hauser, 1995; Hunter, 1998). Less conspicuous drivers, such as patterns of organization, permit the phenotype to evolve as quasi-independent units, promoting morphological (and any associated functional) diversity (Schlosser & Wagner, 2004; Vermeij, 2015). Modularity is such an organizational pattern whereby developmentally and/or functionally related traits coevolve as subunits or "modules" (Schlosser & Wagner, 2004; Wagner, 1996; Zelditch & Goswami, 2021). Modularity can increase efficiency within a system (Olson & Miller, 1999), circumvent trade-offs that would otherwise constrain morphological evolution (Felice et al., 2018; Sanger et al., 2012; Wagner, 1996; Winther, 2001; Zelditch & Goswami, 2021), and permit exploration of a greater range of phenotypes (Vermeij, 2015). As a result, modularity is widely considered a catalyst of morphological evolution (Larouche et al., 2018; Marroig et al., 2009; Wagner & Altenberg, 1996) and is not necessarily mutually exclusive from innovations (Gatesy & Dial, 1996).

Modularity is evident across many vertebrate groups—mammals, birds, amphibians, and fishes to name a few; perhaps suggesting a fundamental role in generating morphological diversity (Bardua et al., 2020; Drake & Klingenberg, 2010; Evans et al., 2021; Fabre et al., 2020; Felice & Goswami et al., 2018; Porto et al., 2013; Zelditch & Goswami, 2021). For example, the modular nature of the anuran jaw suspensorium facilitated the diversification of various feeding mechanisms across terrestrial and aquatic environments. (Bardua et al., 2020; Nishikawa, 2000). Similar patterns of modularity have also been found in salamanders (Fabre et al., 2020) and caecilians (Bardua et al., 2019), suggesting that these patterns of trait evolution can become phylogenetically conserved (Bardua et al., 2020). So far, modular studies with respect to amphibians have been restricted to discrete structures that appear functionally independent such as the cranium or limbs (Bardua et al., 2020; Uroševi ć et al., 2024). However, given the multifunctionality of structures like the head (Davic & Welsh, 2004), a broader analysis of modularity across the phenotype may further our understanding of modularity and its consequences.

The lungless salamanders (Plethodontidae) comprise the largest family of salamanders with approximately 519 species including the major genera *Plethodon*, *Eurycea*, *Desmognathus*, and *Bolitoglossa* (AmphibiaWeb, 2026; Dunn, 1926; Wake, 1966). Members of this family are united by the absence of lungs or gills as adults (Dunn, 1926). Their range spans from the southwestern coast of British Colombia to the Andes Mountains in southern

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Bolivia, but their highest species richness is found in the eastern United States, within the southern Appalachian Mountains (Kozak & Wiens, 2010; Kozak, 2017). Plethodontids in this region also exhibit incredible behavioral, life history, and microhabitat diversity (Hantak et al., 2021; Kozak, 2017; Wake & Hanken, 1996; Weaver et al., 2020). Dusky salamanders (*Desmognathus*; $n = 39$ *spp.*) have a robust feeding repertoire including muscle powered tongue projection and a strong bite enabled by a synapomorphic ligament suite (Deban & Richardson, 2017; Schwenk & Wake, 1993; Wake, 1966). Similarly, *Eurycea* ($n = 32$ *spp.*) have complex life histories, paired with a spectrum of microhabitat specialization, ranging from fully aquatic paedomorphs in caves to semiaquatic species along stream margins and springs (Wake, 1966). Owing to this ecological diversity, both *Desmognathus* and *Eurycea* have been touted as adaptive radiations (Bruce, 1996; Ryan & Bruce, 2000). By contrast, *Plethodon* ($n = 65$ *spp.*) exhibits species diversification that far outpaces morphological diversification (Adams et al., 2009), leading to the widely held notion that they represent a nonadaptive radiation (Highton et al., 2012; Kozak et al., 2006b). These contrasting evolutionary outcomes among subclades—high and low ecological, functional, and life history diversity—makes plethodontids an ideal study system to investigate mechanisms that generate biodiversity.

In this study, we evaluate the role of modularity during the morphological evolution of Appalachian lungless salamanders. First, we examine the morphological diversity of the group by measuring major morphological aspects of the phenotype: head, trunk, tail, limbs, hands/feet, and digits. Second, we test whether (i) Appalachian species and the three major subclades *Plethodon*, *Eurycea*, and *Desmognathus* express signals of modularity between spatially and functionally related morphological characters using a model fitting framework and Covariance Ratio test; (ii) if modules have different evolutionary rates estimated with multiple Bayesian models; and (iii) if modules are evolutionarily coupled or decoupled (i.e., have correlated or uncorrelated evolutionary rates) using additional phylogenetic comparative methods. We hypothesize that modularity underlies the morphological evolution of plethodontids by enabling different regions of the phenotype to evolve independently and at different rates.

Methods

Study group

Plethodontids—the lungless salamanders—are united by the absence of lungs as adults, with a clade age estimate between 90 and 100 million years (Bonett & Blair, 2017; Stewart & Wiens, 2025). Ten of the 28 genera are represented in the Appalachian Mountains and eastern United States, a hotspot for plethodontid richness, ecology, and phenotypic diversity (Kozak, 2017). In this study, we define an Appalachian species as one whose distribution includes a state where the Appalachian Mountains or their foothills occur. This approach includes some species that live adjacent to mountainous regions (e.g., *Phaeognathus* in coastal Alabama) but excludes major discontinuous regions such as the Ozarks and Edwards Plateau in Texas. Four of these genera are monotypic with highly specialized morphology and ecology, such as *Phaeognathus hubrichti*, which is primarily fossorial, either creating or co-opting underground burrows with a "worm-like" elongated body and short limbs (Highton, 1961). *Urspelerpes brucei*, *Hemidactylium scutatum*, and *Stereochilus marginatus* are three small species, which are also monotypic. The former two are primarily terrestrial while *S. marginatus* is generally found in slow-moving blackwater streams and swamps (Camp et al., 2009; Stevenson, 2008). *Aneides aeneus* is a habitat specialist residing in rock crevices and outcroppings with a dorsoventrally flattened body and long limbs (Cope & Packard, 1881). *Pseudotriton* contains two semiaquatic species, either living at the head of muddy burrows (*P. montanus*), or under rocks in shallows springs and streams (*P. ruber*; Bruce, 1975; Petranka, 1998). Both species use an ambush strategy for hunting, sitting at the entrance to their burrow or cover while waiting for prey to pass, although, *P. ruber* is also fond of searching the surrounding riparian zone for food, especially at night (Petranka, 1998). *Gyrinophilus* has four species known as the spring salamanders. Three of these species are troglobitic, residing in caves and emergent springs while the fourth species, *G. porphyriticus*, is an avid hunter in mountain streams, primarily feeding on insects, crayfishes, and other salamanders (Petranka, 1998). The last three genera—*Plethodon*, *Eurycea*, and *Desmognathus* are the most species-rich in the Appalachians. *Plethodon* includes many large-bodied, direct developing species complexes such as the slimy salamanders (*P. glutinosus*, *P. mississippi*, *P. grobmani*, etc.) and the gray-cheeked salamanders (*P. amplus*, *P. meridianus*, *P. metcalfi*, and *P. montanus*), among others (Highton, 1995). *Eurycea* is primarily composed of either semiaquatic or mostly terrestrial species with long trunks, limbs, and tails, as well as some extreme phenotypes such as the pigment loss, eye loss, and paedomorphism found in *E. wallacei*, the Georgia blind salamander (Carr, 1939; Petranka, 1998). Many more cave-adapted euryceans, not included in this study, inhabit the Ozark Mountains and Edwards Plateau. *Desmognathus* exhibit a wide range of microhabitat preferences and life histories from biphasic, fully aquatic (e.g., *D. marmoratus*) to fully terrestrial direct developers (e.g., *D. aeneus)*, united by a synapomorphic, cranial ligament suite (Bruce, 1996; Wake, 1966). Importantly, our study is based on extant species, and therefore, does not consider the diversity of plethodontid salamanders that may have been lost to extinction (Clark, 1985; Min et al., 2005; Venczel & Sanchíz, 2005).

Morphological traits

We measured 383 individuals representing 77 species (three to five individuals per species) distributed throughout the Appalachian Mountains, foothills, and surrounding plains at the Smithsonian Museum Support Center (Figure 1; Data 1; Mullins & Burress, 2026). We measured 30 linear dimensions of the head, trunk, limbs, and tail that are either known to be or are potentially functionally relevant for feeding and/or locomotion in salamanders (Deban & Wake, 2000; Edgington & Taylor, 2019; Wainwright & Reilly, 1994; Wake & Dresner, 1967). These measurements included: snout–vent length (SVL), intergirdle distance, head length, width, and depth, mouth length and width, snout length and depth, internasal distance, eye diameter, "neck" length, trunk depth, pectoral width, pelvic width, humerus length and width, radius length and width, third fore digit

length, fore palm length, femur length and width, tibia length and width, third hind digit length, hind palm length, tail length, width, and depth (see Table S1 for detailed descriptions of each measurement). All measurements were taken by a single individual using digital calipers to a precision of 0.001 cm. Since linear dimensions scale strongly with body size, we calculated phylogenetic residuals with Brownian motion (BM) by regressing ln-transformed linear variables against ln-transformed SVL using the phyl.resid function in *phytools* (Revell, 2012). During this procedure and subsequent phylogenetic comparative methods, we used an existing time-calibrated phylogeny of Plethodontidae based on three mitochondrial and four nuclear genes (Figure 1; Bonett & Blair, 2017). This phylogeny does not encompass the recent inflation of species diversity within *Desmognathus* from the Appalachians and adjacent plains (Pyron & Beamer, 2022a, 2022b; Pyron et al., 2022); however, the group's highly conserved morphology (Schwenk & Wake, 1993) suggests we likely sampled a representative subset of their morphological diversity. We then visually explored phenotypic variation among species using Principal Components Analysis employed with the prcomp function in R (R Core Team, 2026). We calculated the morphological disparity of the three major genera (*Desmognathus*, *Eurycea*, and *Plethodon*) using the disparity function in *geiger* (Harmon et al., 2008). During this procedure, we used the phylogenetic residuals as input and disparity was calculated as the average squared Euclidean distance. Since these groupings are clades (hence, no replication), we did not formally account for phylogeny or test for significant differences among these groups. Owing to this statistical limitation, we also limit our interpretation of disparity.

Modularity tests

We tested for evolutionary, functional modularity across the salamander body plan for all Appalachian plethodontids as well as separately for the three major clades (*Plethodon*, *Eurycea*, and *Desmognathus*). For each taxonomic group, we tested a total of 12 modular hypotheses (Figure 2). Modules were defined by anatomically cohesive groups of traits based on form and function, resulting in four broad groupings—the "head," "trunk," "limbs," and "tail," with additional models in which those regions were intuitively pooled. Since the widths of the pectoral and pelvic girdles are part of the appendicular skeleton but also define a dimension of the trunk, we tested alternative models in which the girdles were pooled with the trunk or with the limbs. In total, we tested twelve models with four levels of complexity: (i) the null hypothesis, which is a model with no modularity; (ii) three models with two modules—either the head and "body" (trunk + limbs + tail), the axial (head + trunk + tail) and appendicular (limbs) skeletal elements, or the "body" (head + trunk) and appendages (limbs + tail); (iii) two models with three modules, which are identical to the latter two models above but with the head as a separate, third module; and (iv) one model with four modules (head + trunk + limbs + tail). The final five models are the same as the latter five but with the girdles treated in the two alternative ways detailed above (see Figure 2).

We used two methods to test for modularity: the covariance ratio (CR; Adams, 2016) and EMMLi (Bardua et al., 2020; Goswami & Finarelli, 2016). EMMLi utilizes a model-fitting framework but has been criticized for preferring overparameterized models; therefore, it is commonly used in conjunction with the CR (Adams & Collyer, 2019; Duclos et al., 2025; Zelditch & Goswami, 2021). EMMLi is a maximum-likelihood approach that uses the Akaike Information Criterion (AICc) to compare the relative fit of different modular hypotheses (Goswami & Finarelli, 2016). Alternatively, the CR quantifies the degree of modularity in an a priori model and tests it against a null model of randomly partitioned traits (Adams, 2016). All 12 models were assessed using both the phyloEmmli function in the package *EMMLi* (Bardua et al., 2020; Goswami & Finarelli, 2016), and the phylo.modularity and compare.CR functions in *geomorph* (Adams & Collyer, 2016; Adams et al., 2024). The phylo.modularity function performs the CR analysis for a given model while also accounting for phylogenetic nonindependence of the trait data. Additionally, it provides the CR coefficient, is insensitive to sample size, and determines whether the model is statistically different than a null model with randomly partitioned traits (i.e., no modularity). Similarly, the compare.CR function performs pairwise comparisons of the effect sizes of different models to determine the best fit (lowest effect size) and whether it is significantly different from the null model. When the best model in EMMLi was different than the best model in the CR analysis, we used the pairwise comparisons implemented in the compare.CR function to determine whether the EMMLi model had significantly different support than the CR model. If the two models were not significantly different in their support, we reported both models. In two cases (*Eurycea* and *Desmognathus*), multiple models from EMMLi had nearly equal support (ΔAICc < 2), therefore, among those equally supported models, we chose to emphasize the one that most closely agreed with the CR best-fit model (the full breakdown of the EMMLi results can be found in Tables S2–S5). Lastly, to interpret the strength of the modular signal in each best fit model, we used the CR coefficient and effect sizes from the *geomorph* outputs. Additionally, we used the estimated rho (ρ) correlation coefficients from EMMLi to assess the within and between module integration.

Rates of phenotypic evolution

To assess if modules differ in their evolutionary rate and are evolutionarily correlated, we estimated the rates of phenotypic evolution for each of the three (or four) modules as defined by the best-supported models according to CR (i.e., head, body, and limbs) and EMMLi (i.e., head, trunk, limbs, and tail) for the Appalachian species. Here, we cautiously interpret differences in evolutionary rate among modules, as modules vary in their dimensionality (i.e., number of traits) and trait variance such that rates are expected to be largely reflective of differences in variance. We estimated mean rates for each module in two complementary ways. First, we employed the compare.multi.evol.rates function in *geomorph* (Adams & Collyer, 2016; Adams et al., 2024). As an additional estimate of rates, we used a multivariate relaxed-BM model of evolution (May & Moore, 2020) implemented in RevBayes (Höhna et al., 2016). This method relaxes assumptions of BM in several important ways—by letting the evolutionary rate vary across the phylogeny and through time (i.e., by permitting rate shifts among branches) and accounts for the correlated history of the continuous characters (May

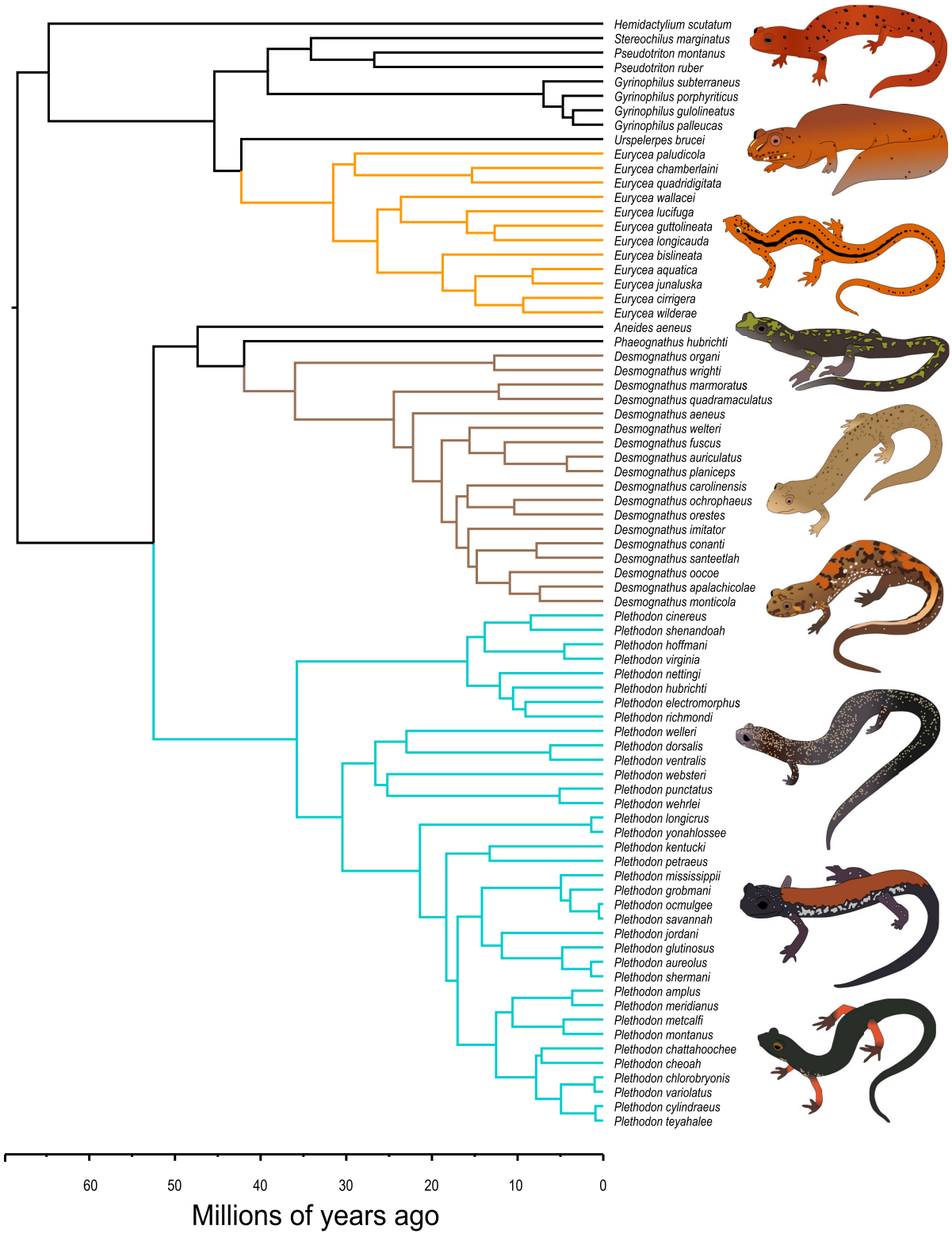

Figure 1. Taxon sampling of Appalachian lungless salamanders. The three focal clades, Brook Salamanders (*Eurycea*), Dusky Salamanders (*Desmognathus*), and Woodland Salamanders (*Plethodon*) are highlighted in yellow, brown, and blue respectively. Illustrations depict an adjacent species. Phylogeny adapted from Bonett & Blair (2017).

& Moore, 2020). The Markov chain Monte Carlo ran for 1,000,000 generations with a 10% burn-in for all iterations. We estimated the rates in two fundamentally different ways: (1) with a random local clock (RLC) in which evolutionary rate is inherited with a chance of a rate shift and (2) an uncorrelated lognormal clock (UCLN) in which the evolutionary

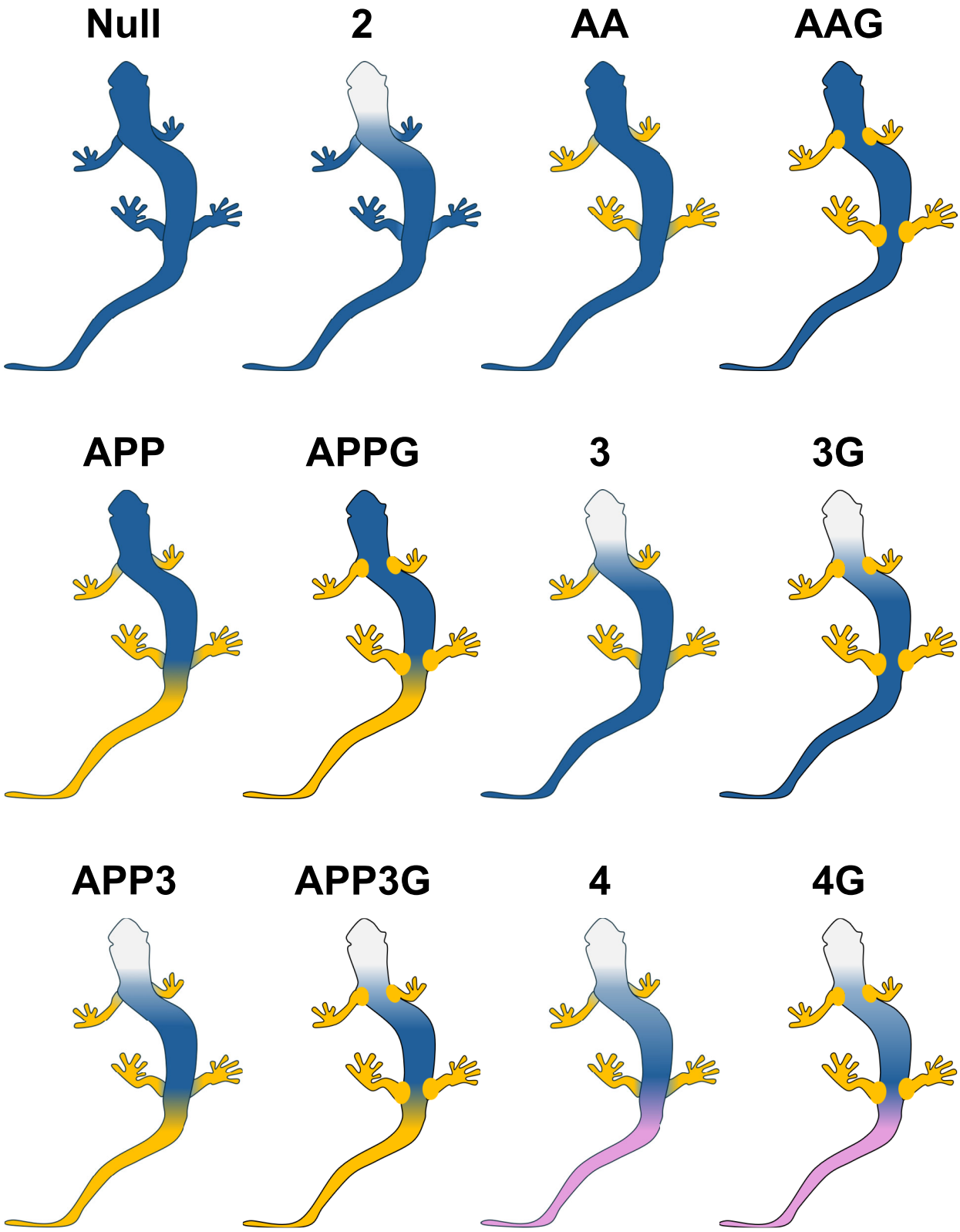

Figure 2. Hypotheses of functional modularity in Appalachian plethodontid salamanders. Models that end with "G" have the girdles included with the limbs, which are otherwise included with the trunk. AA = axial and appendicular; APP = appendages.

rate is estimated separately for each branch. We evaluated sensitivity to the user-specified prior number of rate shifts for the RLC model, by repeating the analyses with priors of 1, 25, and 50 rate shifts. To determine which modular rates of evolution were (de)coupled, we tested for correlations between the ln-transformed tip rates of each module using phylogenetic generalized least squares (Burress & Hart, 2024; Rabosky et al., 2013; Revell, 2010). Tip rates, or

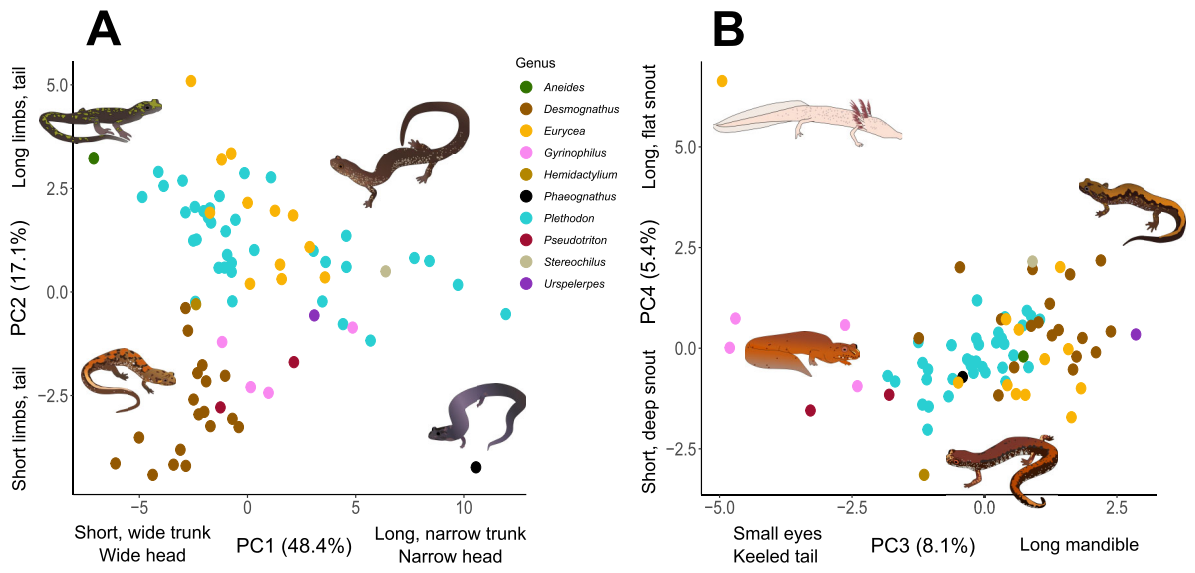

Figure 3. Morphological variation among 77 species of Appalachian lungless salamanders. Points represent species means from principal components. Illustrations depict an adjacent species. Text along the extremes of axes indicate trait change associated with that direction.

Table 1. Functional modularity in Appalachian plethodontid salamanders and focal subclades. See the section "Methods" and Figure 2 for descriptions of model abbreviations. See Tables S2–S5 for parameters and support for every tested model. Note that the reported *p*-values are from the phylo.modularity function and differ slightly from those shown in Tables S6–S9, which use the compare.CR function.

Clade	Method	Best-fit model	CR coefficient	Effect Size	*p*-value
Appalachian	EMMLi	4G	0.914	−2.347	.008
	CR Test	3G	0.736	−3.607	.001
Plethodon	EMMLi	4G	0.857	−3.107	.003
	CR Test	4	0.759	−4.363	.001
Eurycea	EMMLi†	3	0.857	−2.454	.003
	CR Test	3G	0.833	−2.478	.007
Desmognathus	EMMLi†	4	1.090	−1.226	.105
	CR Test	4	1.090	−1.226	.105

Footnote: † indicates that the posterior probability was less than 0.95.

species-specific rates of evolution, reflect relatively recent evolution (Title & Rabosky, 2019).

Results

Phenotypic variation

The major axes of phenotypic variation among species (PC1 = 48.4%; PC2 = 17.1%) are elongation of the head, trunk, limbs, and tail, ranging from short, stout species (e.g., *Desmognathus*) to elongate, gracile species (e.g., *Phaeognathus*; Figure 3A). Other notable axes of phenotypic variation include mandible length, eye diameter, tail keel (PC3 = 8.1%), and snout length and depth (PC4 = 5.4%; Figure 3B). Some genera exhibit distinct phenotypes. For example, *Desmognathus* and *Gyrinophilus* have phenotypes characterized by short, stout bodies, limbs, and tail (Figure 3A and B). *Phaeognathus* have long, slim bodies with short limbs (Figure 3A). *Aneides* have short bodies, long limbs and tail, and a wide dorsoventrally compressed head (Figure 3A). *Eurycea* exhibit more variation and overlap with other genera, but include individual species with novel and extreme phenotypes such as *E. wallacei* with a strongly keeled tail; long, shallow snout; and small vestigial eyespots (Figure 3B). *Plethodon* have the widest distribution in morphospace, overlapping with many other genera, varying in their relative body elongation and limb lengths (Figure 3A). Of the focal subclades, *Desmognathus* exhibit approximately half the morphological disparity (0.823) of *Eurycea* and *Plethodon* (1.474 and 1.472, respectively).

Evolutionary modularity

Support for the alternative modularity hypotheses and the strength of modularity differed between methods and among the Appalachian group and *Plethodon*, *Eurycea*, and *Desmognathus* subclades (Table 1). Each focal group had at least one statistically significant best-fit model when accounting for phylogenetic nonindependence, except *Desmognathus* for which none of the models were statistically significant (Table 1). In the Appalachians, *Plethodon*, and *Eurycea*, EMMLi and CR preferred different best-fit models, whereas in *Desmognathus*, both methods agreed on the best-fit model (Table 1).

Across all Appalachian species, 10 of the 12 models were statistically significant (Table S6). The model with the highest support based on EMMLi had four modules (head, trunk, limbs/girdles, and tail) and a relatively low but significant degree of modularity (p = .008, CR = 0.914,

z = -2.347; Figure 4A). By contrast, the model with the highest support based on CR had three modules (head, body, and limbs/girdles) and the strongest modular signal of any focal group, based on the CR coefficient ($p = .001$, CR = 0.736, $z = -3.607$; Figure 4B).

In *Plethodon*, ten of the twelve models were statistically significant (Table S7). The model with the highest support based on EMMLi, which was the same as the Appalachian group, had four modules (head, trunk, limbs/girdles, and tail) and a relatively strong degree of modularity ($p = .003$, CR = 0.857, $z = -3.107$; Figure 4C). The model with the highest support based on CR was very similar, also including four modules but changing the placement of the girdles (head, trunk/girdles, limbs, and tail) and had the strongest modular signal based on the z-score ($p = .001$, CR = 0.759, $z = -4.363$; Figure 4D).

In *Eurycea*, only five of the twelve models were statistically significant (Table S8). Additionally, the ΔAICc was similar (i.e.,; <2) to the models with the highest support according to EMMLi; therefore, we favor the model that agrees most closely with the best-fit model based on CR, which had three modules (head, body/girdles, and limbs) and a moderate degree of modularity ($p = .003$, CR = 0.857, $z = -2.454$; Figure 4E). The best-fit model based on CR was very similar, differing only by the placement of the girdles (head, body, and limbs/girdles). The signal and support were also nearly identical ($p = .007$, CR = 0.833, $z = -2.478$; Figure 4F).

In *Desmognathus*, the best-fit model had four modules (head, trunk/girdles, limbs, and tail), yet had a nonsignificant degree of modularity ($p = .105$, CR = 1.090, $z = -1.226$; Table 1), as did all other models (Table S9).

Across the three focal groups that exhibit modularity—Appalachian, *Plethodon*, and *Eurycea*—the limbs were consistently the most integrated module ($\rho = 0.42$–0.47; Tables S10-S15). The head and tail modules were also relatively strongly integrated, especially in *Desmognathus* where, though not statistically significant, the tail "module" had the strongest correlation of any within or between-module pairwise comparisons ($\rho = 0.66$; Table S16). Apart from this value, none of the within-module correlations were exceptionally strong within any group, however, they were all consistently higher than the between-module correlations in the Appalachian, *Plethodon*, and *Eurycea* focal groups (Tables S10-S15).

Evolutionary rates

The rates of module evolution were highly correlated and near 1-to-1 between the RLC and UCLN model estimates ($R^2 = 0.86$; Figure S1) and, consequently, estimated mean rates were consistent among runs with different priors (Figure S2). Across all Appalachian species, modules varied about 1.5-fold in their mean evolutionary rate estimated in RevBayes (Figure 5) and about 2.7-fold estimated in geomorph. Both methods supported the same rank order of (fastest-to-slowest) tail, limbs, head, and trunk (model favored by EMMLi) and body, limbs, head (model favored by CR); however, these differences were not significantly different than a single-rate model ($p > .05$). Tip rates were significantly correlated among modules, with one exception—the limbs were evolutionarily decoupled from all other modules except the head (Tables S17-S19).

Discussion

Modularity is hypothesized to promote morphological evolution by increasing the efficiency of the anatomical system (Olson & Miller, 1999), allowing subsets of the phenotype to circumvent trade-offs that might constrain the evolution of other regions (Felice et al., 2018; Sanger et al., 2012; Wagner, 1996; Winther, 2001; Zelditch & Goswami, 2021), and permit exploration of a greater range of phenotypes (Vermeij, 2015). We found that salamander morphology exhibits a modest degree of modularity across all Appalachian species, likely due to heterogeneity among major subclades, which varied from somewhat strongly modular to nonmodular (Table 1). Modularity does not result in modules evolving at different rates (Figure 5) and species-specific evolutionary rates were mostly coupled between modules (Tables S17–S19). Taken together, these results suggest that modularity underlies the morphological diversity across Appalachian salamanders by permitting different regions of the phenotype to evolve independently, but those modules largely responded in concert to ecological opportunity.

Modularity as a catalyst of morphological evolution

In the Appalachian Mountains, lungless salamanders often dominate the terrestrial, subterranean, and aquatic ecosystems as some of the most ecologically important and abundant vertebrate predators (Burton & Likens, 1975; Davic & Welsh, 2004; Hairston, 1987). There is a strong correlation between ecomorphology and microhabitat use within genera (e.g., *Desmognathus*; Kozak et al., 2005; and *Eurycea*; Edginton & Taylor, 2019), but this relationship becomes far less pronounced among genera (Blankers et al., 2012), perhaps highlighting lineage-specific relationships between morphology and function. The degree of modularity varied significantly among major lineages of lungless salamanders. On one extreme, Dusky Salamanders (*Desmognathus*) do not exhibit modularity (Table 1). This genus has a unique phenotype associated with a stout trunk and head with short appendages but occupy a limited range of morphospace (Figure 3), highlighting the morphological similarity among congeners. This genus is considered an adaptive radiation, but the nature of this diversity is related to microclimate and broad use of the aquatic-to-terrestrial microhabitat dimension (Bruce, 1996; Kozak et al., 2005; Weaver et al., 2020), rather than extensive morphological diversification. The physiological nature of their adaptive radiation may also explain why body size is strongly correlated with microhabitat use (Kozak et al., 2005) due to its relationship with surface area, and by extension, processes such as water loss and gas exchange (Baken et al., 2020; Riddell et al., 2018). This lineage is innovation laden (Burress et al., 2026), but most are small modifications to the osteology at the junction between the anterior vertebrae and cranium or involves soft tissues such as ligaments (Deban & Richardson, 2017; Schwenk & Wake, 1993; Wake, 1966). Therefore, the apparent morphological similarity among *Desmognathus* species may be due to a combination of their innovations being synapomorphic (Schwenk & Wake, 1993), much of their diversity being related to microclimate (Weaver et al., 2020), and a lack of modularity that might otherwise facilitate more pronounced morphological evolution (Table 1).

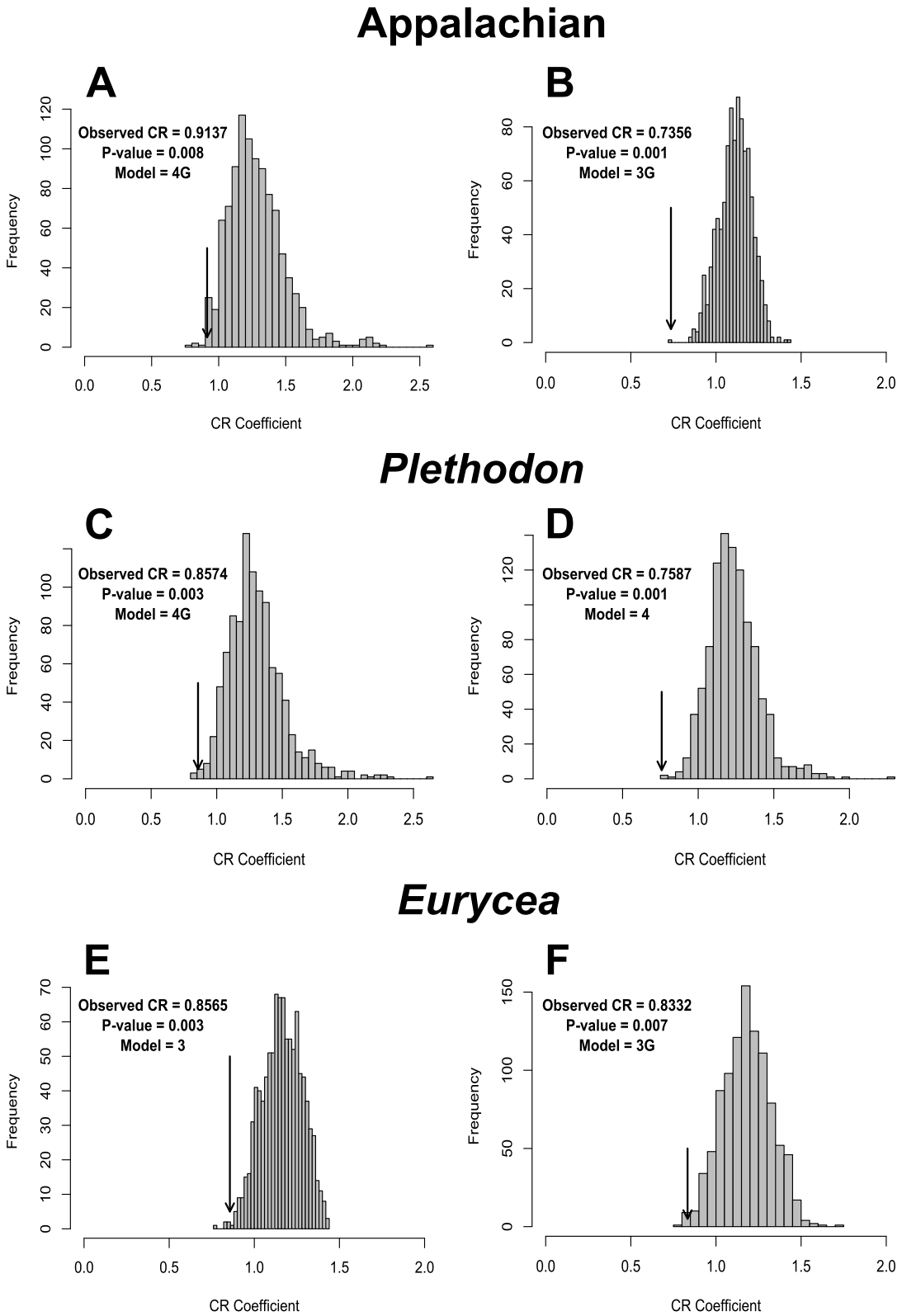

Figure 4. Covariance ratio distributions for the best fitting modularity hypotheses based on EMMLi (A and C), the Covariance Ratio test (B and D), or both (E and F), for each labelled group. Arrow depicts the position of the observed covariance ratio relative to the distribution of values from a null model of randomly partitioned traits. Note that *Desmognathus* is not shown because it did not exhibit significant modularity. CR coefficients and P-values were calculated via the phylo.modularity function in *geomorph* (Adams & Collyer, 2016).

On the other extreme, *Eurycea* and *Plethodon* exhibit considerable morphological variation (Figure 3) paired with significant modularity (Table 1). *Eurycea* is the third largest clade in the Appalachians, is considered an adaptive radiation (Ryan & Bruce, 2000; Wray & Steppan, 2017), and exhibits elevated rates of morphological evolution (Bonett &

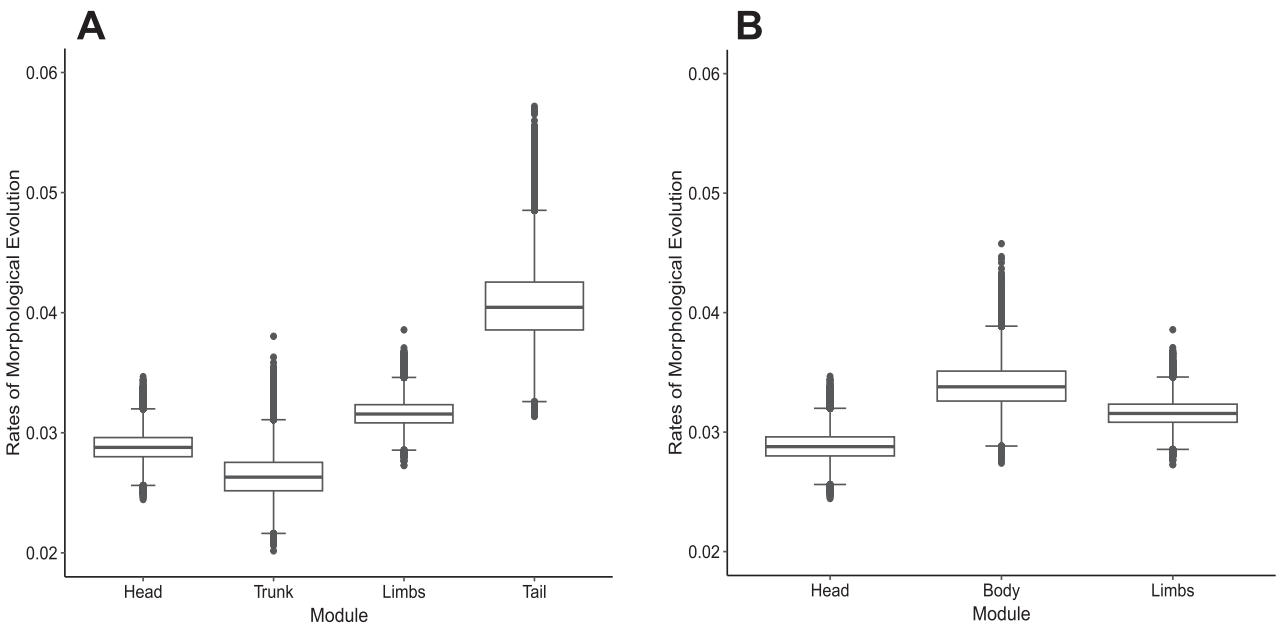

Figure 5. Rates of morphological evolution among functional modules, as identified as the best-supported model based on EMMLi (A) and CR (B). Boxplots depict the mean rate for each module, but their rates were not significantly different ($p > .05$). The "body" refers to the trunk + tail module. See the section "Methods" and Table 1 for full descriptions and support for each model. Rates were estimated with an RLC model (see Figure S1 for comparison with a UCLN model). Results from the run with a prior of 50 rate shifts is shown, see Figure S2 for comparison of runs with different priors.

Blair, 2017; Wray & Steppan, 2017). Unique compared to the other major clades (*Desmognathus* and *Plethodon*), *Eurycea* has independently colonized caves throughout karst regions of the southeastern United States, often associated with paedomorphy (Bendik et al., 2013; Bonett & Blair, 2017; Tovar et al., 2025). Cave salamanders often have dramatic eye and pigment loss, elongated bodies, limbs, and digits, and wider heads and shorter tails, relative to other plethodontid species (Edgington & Taylor, 2019). *Eurycea* had a strong modular signal across three partitions—the head, body, and limbs, which may facilitate adaptation of those regions to cave environments. In particular, *Eurycea* exhibit considerable variation in tail morphology, including some of the shortest (*E. wallacei*) and longest tails (*E. longicauda*) as well as strongly keeled (*E. wallacei*) to near round tails (*E. wilderae*). *Eurycea* also tended to have long appendages relative to their body length (Figure 3). This spindly phenotype may facilitate traversing long terrestrial and aquatic distances, which many semiaquatic species are known to undertake (Bruce, 1986; Kozak et al., 2006a; MacCulloch & Bider, 1975). Although much of their ecological diversity is intrinsically linked to their complex life histories (Bonett & Blair, 2017), our results point to modularity as an additional mechanism underlying their putative adaptive radiation.

An old, rapid radiation followed by morphological stasis is epitomized by Woodland Salamanders (*Plethodon*; Kozak et al., 2006b; Mahoney, 2001). Consequently, this clade is considered a classic nonadaptive radiation due to its extensive genetic differentiation yet highly conserved morphology and ecology (Camp & Wooten, 2016; Kozak et al., 2006b; Smith et al., 2018; Wake et al., 1983). Here, we found that *Plethodon* have the strongest signal of modularity, including four modules—the head, trunk, limbs, and tail (Table 1) and exhibit considerable morphological variation, on par with *Eurycea*, and nearly twice that of *Desmognathus*, two lineages considered adaptive radiations (Bruce, 1996; Kozak et al., 2005; Ryan & Bruce, 2000; Weaver et al., 2020; Wray & Steppan, 2017). This apparent paradox warrants further exploration and highlights how much of *Eurycea*'s phenotypic (and ecological) diversity is directly tied to their biphasic lifecycle (Bonett & Blair, 2017; Ryan & Bruce, 2000) rather than the morphological diversity of metamorphosed adults. Most of the disparity among *Plethodon* is restricted to the major axis of variation—elongation of the trunk and tail (PC1; Figure 3A). This pattern may superficially inflate their disparity, while they have not explored many other axes of variation. For example, most *Plethodon* are largely terrestrial, with some species reasonably considered fossorial or saxicolous (Baken & Adams, 2019; Blankers et al., 2012; Burress et al., 2026). Some species are, in part, opportunistic occupants of caves (e.g., *Plethodon glutinosus*; Himes et al., 2004; Zigler et al., 2020), but they lack any adaptations for cave life, such as pigment or eye loss (Edgington & Taylor, 2019). Further, *Plethodon* are direct developers, and therefore, lack an obliged interaction with nonterrestrial environments (Bonett & Blair, 2017). The existence of hidden dimensions of diversity within lungless salamanders, including *Plethodon*, has been noted by others (Camp & Wooten, 2016), but these instances have mostly been attributed to divergence in body size (Carr, 1996; Kozak et al., 2006b; Wake et al., 1983) rather than specific aspects of the phenotype that are less multifunctional. Modularity commonly holds a place in the evolutionary history of complex organisms, regardless of how ecologically disparate the resulting lineages become (Felice et al., 2018; Zelditch & Goswami, 2021). However, their morphological disparity and extent of modularity

hints at *Plethodon* harboring more diversity than previously appreciated.

Ecological consequences of modularity

The pattern of modularity across all Appalachian species was likely influenced by the major clades, as the two best-fit models were very similar to those of *Plethodon* and *Eurycea* (Table 1; Tables S2–S4 and S6–S8). Similarly, the lack of modularity in the third major subclade, *Desmognathus*, likely reduced the modular signal across all Appalachian species relative to *Eurycea* and *Plethodon* (Table 1). The best-fit models were a four-module system that includes the head, trunk, limbs/girdles, and tail (EMMLi) or a three-module system that includes the head, body, and limbs/girdles (CR). In both cases, the head and the limbs were separate modules, possibly because traits within these modules were highly integrated (Tables S10–S16). In lungless salamanders, the head is multifunctional. Principal among those functions is prey capture. Since most plethodontids are generalist predators, head size and gape are important determinants of diet and successful prey capture (Adams et al., 2009; Kryzsik, 1979; Petranka, 1998). The head can also play a central role in locomotion and access to novel microhabitat when species engage in digging behavior (Schwenk & Wake, 1993). Although one species is highly fossorial year-round (*Phaeognathus hubrichti*; Dodd, 1991), nearly all Appalachian plethodontids spend some fraction of the year underground in secondhand or self-made burrows, suggesting this locomotor utility of the head is likely far reaching (Davic & Welsh, 2004). Further, numerous chemosensory systems are centralized on the head, including the nasolabial grooves used in olfaction, and in males, the mental gland used in courtship (Brown & Martof, 1966; Houck & Sever, 1994). The limbs also serve various essential locomotor functions throughout the life history of salamanders. Biphasic species migrate for food and breeding, with some traveling over 100 m from their home stream at a time (e.g., *Eurycea*; MacCulloch & Bider, 1975; Pauley et al., 2000). This movement also contributes to the ecological significance of salamanders on community stability, as migrating between terrestrial and aquatic ecosystems can strongly impact food web dynamics by introducing allochthonous resources to either habitat (Polis et al., 1996). Limb morphology plays an important role in the hunting strategies of various species, such as *Eurycea lucifuga*, which mature and hunt around limestone caves, or the many arboreal *Bolitoglossa* that feed among trees and tree branches in the tropics (Wake & Brame, 1963; Ringia & Lips, 2007). Finally, predator evasion through sprinting or burrowing is a common tactic paired with tail autonomy, glue-like slime secretions, or aposematism. (Brodie et al., 1979; Full et al., 1988; Wake & Dresner, 1967).

The modules did not exhibit different evolutionary rates (Figure 5), contrary to expectations, as mosaic evolution is considered an adaptive feature of modularity (Felice et al., 2018; Sanger et al., 2012; Wagner, 1996; Winther, 2001; Zelditch & Goswami, 2021). The trunk's ability to diversify, in particular, is likely constrained in the face of water loss and gas exchange, given the group's exclusive use of cutaneous respiration as adults coupled with the trunk's disproportionate impact on surface area (Baken et al., 2020; Riddell et al., 2018). This may explain why the trunk is coupled with most other modules (Tables S17–S19) and its decoupling from the limb module may reflect broader decoupling of physiological and locomotion-related morphological traits (Bodensteiner et al., 2024). The rates of tail evolution were significantly correlated with all other modules except for the limbs (Tables S17–S19). The evolutionary decoupling of the limbs and tail was unexpected given that the two are often functionally related to locomotor performance (Losos, 1990), and therefore, might respond in concert to ecological opportunity (i.e., exhibit elevated rates). On the other hand, their evolutionary decoupling may arise due to interactions with microhabitat. On land, the limbs are responsible for locomotion such as while climbing and clinging to surfaces (Baken & Adams, 2019; Baken & O'Donnell, 2021), whereas in water forward propulsion is driven by the tail (Marvin, 2013). Given the fraction of Appalachian salamanders that are biphasic or otherwise semiaquatic (Baken & Adams, 2019; Blankers et al., 2012; Weaver et al., 2020), this functional tension may be far-reaching, enough to promote the evolutionary decoupling of the limbs and tail (Shaffer er al. et al., 1991). By contrast, the head module was evolutionarily coupled with all other modules (Tables S17–S19). This suggests that the head may broadly respond to ecological opportunity in unison with other regions of the phenotype. In other words, if ecological opportunity spurs diversification of the limbs or tail, the head often responds similarly, perhaps due to its potential to be functionally ensnared with locomotion (i.e., playing a part as terrestrial and aquatic species engage in burrowing; Parra-Olea & Wake, 2001; Schwenk & Wake, 1993).

Adaptive radiations tend to exhibit bursts of phenotypic evolution and/or speciation, although those trends are not always mutually inclusive (Burress & Hart, 2024; Glor, 2010; Kozak et al., 2005). This variability in evolutionary outcome is derived from underlying intrinsic and extrinsic drivers and their interconnected nature. From a genetic and developmental basis, organizational patterns such as modularity can ease constraints like functional trade-offs by allowing different regions to evolve semi autonomously, leading to innovations that directly interact with and are affected by extrinsic factors like microhabitat and resource availability. Most amphibian-centric studies have focused on cranial modularity, given its wide-ranging multifunctionality (Bardua et al., 2019, 2020; Fabre et al., 2020). Our study broadens the scope of plethodontid modularity with a novel dataset that spans the entire salamander body plan. We present increased evidence for morphological variation in the widely considered, nonadaptive radiation of *Plethodon*, shrinking the gap between species and morphological diversification in this group (Adams et al., 2009; Kozak et al., 2006b). Conversely, we show that clades known for their ecological diversity (*Desmognathus*) can occupy a limited range of morphologies (Bruce, 1996; Wake, 1966). More broadly, the presence (or lack) of modularity does not neatly correspond to how genera are viewed in terms of the continuum between adaptive and nonadaptive radiation. Modularity underlies the morphological diversity of Appalachian lungless salamanders, and by extension, may underly much of their emergent ecological and functional diversity.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Data for this study have been archived in the Dryad data repository (https://doi.org/10.5061/dryad.gf1vhhn4t).

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Conflict of interest

The authors declare no conflict of interest.

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