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Are rates of species diversification correlated with rates of morphological evolution?

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Some major evolutionary theories predict a relationship between rates of proliferation of new species (species diversification) and rates of morphological divergence between them. However, this relationship has not been rigorously tested using phylogeny-based approaches. Here, we test this relationship with morphological and phylogenetic data from 190 species of plethodontid salamanders. Surprisingly, we find that rates of species diversification and morphological evolution are not significantly correlated, such that rapid diversification can occur with little morphological change, and *vice versa*. We also find that most clades have undergone remarkably similar patterns of morphological evolution (despite extensive sympatry) and that those relatively novel phenotypes are not associated with rapid diversification. Finally, we find a strong relationship between rates of size and shape evolution, which has not been previously tested.

Keywords: diversification; evolution; morphology; phylogeny; salamanders; speciation

1. INTRODUCTION

What is the relationship between rates of species diversification and rates of morphological evolution? In general, one might expect that rates of species diversification (rate of speciation − rate of extinction) and evolutionary changes in morphology should be positively correlated. Indeed, several evolutionary theories implicitly predict this relationship. For example, the ecological theory of adaptive radiation predicts that adaptive phenotypic divergence promotes the rapid evolution of large numbers of species, at least initially (Schluter 2000). Similarly, the hypothesis of punctuated equilibrium (e.g. Gould & Eldredge 1977) posits that most phenotypic divergence occurs at speciation events, and some authors have suggested that this may lead to correlated rates of species diversification and phenotypic divergence among clades (e.g. Ricklefs 2004, 2006a), but presumably only if diversification rates vary among clades. Recent studies have shown a general association between ecological divergence and reproductive isolation of sister species (Funk *et al.* 2006), which could lead to adaptive differences in morphology associated with speciation. On the other hand, a relationship between species diversification and morphological change might break down in some cases, such as in non-adaptive radiations (e.g. Kozak *et al.* 2006a) or when morphologically cryptic species proliferate.

Surprisingly, the relationship between rates of species diversification and rates of morphological evolution has never been explicitly tested. However, some previous studies have also addressed closely related topics. Harmon *et al.* (2003) examined the relationship between the timing of species diversification and the evolution of morphological disparity. Others have used phylogenetic approaches to test whether morphological evolution is gradual (related to time) or punctuated (related to speciation; e.g. Mooers *et al.* 1999; Bokma 2002, 2008).

Recently, Ricklefs (2004) found greater morphological variance in clades of birds with more species, a pattern implying an association between rates of speciation and morphological change. Subsequent studies showed that greater morphological variance in species-rich clades may be expected due to their greater age alone (Purvis 2004; Ricklefs 2006a). However, the morphological variance within a clade could be very different from the rate of morphological change, because estimates of within-clade morphological variance or disparity ignore phylogeny (O'Meara *et al.* 2006). For example, imagine two clades, each having 10 small species and 10 large species. In one, the small and large species each belong to two separate subclades, yielding a low overall rate of change in body size across the phylogeny for the group. In the other clade, each small species is the closest relative of a large species, yielding a high overall rate of morphological change. Even though the evolutionary rates would be very different in these two groups, the morphological variance would be identical. Clearly, estimating rates of morphological change and understanding patterns of morphological evolution, require phylogeny-based approaches (see also O'Meara *et al.* 2006).

Plethodontid salamanders are an attractive study system for examining the relationship between rates of morphological change and species diversification among clades, for several reasons. First, previous studies suggest that plethodontids exhibit a diversity of patterns in terms of the relationship between species diversification and morphological change. Some clades appear to be adaptive radiations, seemingly with high rates of morphological

change and species diversification (tropical bolitoglossines; Wake 1987; Wiens 2007). Other clades may represent non-adaptive radiations, seeming to have high rates of diversification and low rates of morphological change (*Plethodon*; Kozak *et al.* 2006*a*). There are also clades that appear to exhibit extensive species diversification and body-size divergence, but not body-shape divergence (*Desmognathus*; Kozak *et al.* 2005), suggesting that these two rates of morphological evolution might be uncoupled. However, these hypotheses have not been explicitly tested, because rates of morphological evolution have not been quantified. Second, the phylogeny of plethodontids is becoming well-resolved, both at higher levels and within genera (e.g. Chippindale *et al.* 2004; Vieites *et al.* 2007; Wiens *et al.* 2007; Kozak *et al.* in press). Finally, the morphological data needed to test these hypotheses are available from the literature (e.g. Wiens & Hoverman 2008) and museum specimens.

In this paper, we address the relationship between rates of morphological evolution and species diversification in plethodontid salamanders. We divide Plethodontidae into 15 carefully selected clades, and estimate diversification rates for these clades based on species numbers and divergence times from a phylogeny containing approximately 50 per cent of the nearly 400 plethodontid species. We obtain morphometric data for 260 species, and estimate rates of morphological evolution in size and shape based on the phylogeny within each clade. We then assess the relationship between diversification rates of clades and their rates of morphological evolution (examining size and shape separately). We also address whether morphological disparity is a reasonable proxy for rates of morphological change, and whether rates of change in size and shape are correlated between clades. Previous authors have examined the relationship between size and shape in a phylogenetic context (e.g. Harmon *et al.* 2005; Collar *et al.* in press), shown different rates of phenotypic evolution among clades for different traits (e.g. Garland 1992), compared divergence in size and shape among species (e.g. Renaud *et al.* 2007), and examined rates of size evolution and rates of shape evolution relative to their geographical area (Harmon *et al.* 2008). However, no study has explicitly compared rates of size evolution to rates of shape evolution among clades.

2. MATERIAL AND METHODS

(a) *Phylogeny and ages of clades*
Plethodontidae contains 391 recognized species (AmphibiaWeb 2008). Our analyses were based on a time-calibrated phylogeny for 191 plethodontid species, including some putative (unnamed) species within *Desmognathus* and *Eurycea* (Kozak *et al.* 2005, 2006*b*). To construct this tree, we started with a phylogeny for 109 plethodontid species (but excluding most tropical bolitoglossines) based on nuclear and mitochondrial DNA sequences (Kozak *et al.* in press). Estimated ages for each clade were based on penalized likelihood analysis in R8S (Sanderson 2003). To incorporate the tropical bolitoglossines, we used the phylogeny from Wiens *et al.* (2007; for 137 species and all recognized genera), but re-estimated the chronogram (using R8S) using the crown-group age of Bolitoglossinae estimated by Kozak *et al.* (in press) as the root age for bolitoglossines. The expanded chronogram for bolitoglossines was then manually grafted onto the tree for 109 plethodontids.

This approach yielded comparable branch lengths throughout the phylogeny, although somewhat different datasets were used to estimate the trees (Wiens *et al.* 2006*b*). The combined tree was then pruned to include only species for which morphometric data were available (see below). In summary, a time-calibrated phylogeny containing 190 species was generated (appendices 1 and 2 in the electronic supplementary material) that included all recognized plethodontid genera (AmphibiaWeb 2008; but note that *Haideotriton* is part of *Eurycea* following Vieites *et al.* 2007 and others). We excluded *Eurycea latitans* because the very short branch length connecting this species to *Eurycea tridentifera* may reflect mitochondrial introgression between these parapatric species (i.e. some individuals are nearly identical in mtDNA, but the species are distinct allozymically; Wiens *et al.* 2003) and strongly influenced estimated rates of morphological evolution. However, *Eurycea* has a rapid rate of morphological evolution, regardless of whether *E. latitans* is included (§3).

(b) *Clade divisions*
Analyses of rates of morphological and species diversification were based on comparisons among clades. From the full phylogeny we selected 15 non-overlapping clades (figure 1), based on the following criteria. First, to avoid problems in estimating rates of change among very few species, only clades with ≥ 4 sampled species were used; clades with fewer species were subsumed into larger clades (given the requirement of monophyly) or otherwise not used. Second, only strongly supported clades were used (bootstrap values $\geq 70\%$; Felsenstein 2004). Third, only clades in which assignment of unsampled species to clades was unambiguous were used (e.g. species belonging to apparently monophyletic genera or species groups). Finally, because different divisions were possible (even using these criteria), most analyses were re-run using an alternative set of clades (tables 3s and 4s in the electronic supplementary material). This alternative division included more species overall, but yielded fewer clades (7 rather than 15).

(c) *Diversification rates*
The diversification rate for a clade can be estimated given the species number and age of the clade. Species numbers for each genus were based primarily on the AmphibiaWeb (2008) database (but including some unnamed species from recent phylogeographic studies; Kozak *et al.* 2005, 2006*a,b*), and ages of clades were taken from the estimated chronogram. We used the method-of-moments estimator for crown groups to obtain diversification rates (eqn (7) of Magallón & Sanderson 2001). Unlike the standard maximum likelihood estimator (e.g. the log of clade species richness divided by clade age), this estimator does not require assuming that the extinction rate is negligible, and thereby avoids some potential biases in estimating diversification rate (Magallón & Sanderson 2001). We used a broad range of values for relative extinction rates (0.00, 0.45 and 0.90), given that the actual values are unknown. We acknowledge that this approach assumes that relative extinction rates are similar across all clades in each analysis, although we vary these rates between analyses. However, violations of the assumption of equal relative rates should not invalidate attempts to estimate and compare overall diversification rates, even if they do make it difficult to disentangle the relative contributions of speciation and extinction rates to the overall diversification rate, which we do not attempt to do (Ricklefs 2006*b*). Diversification rates
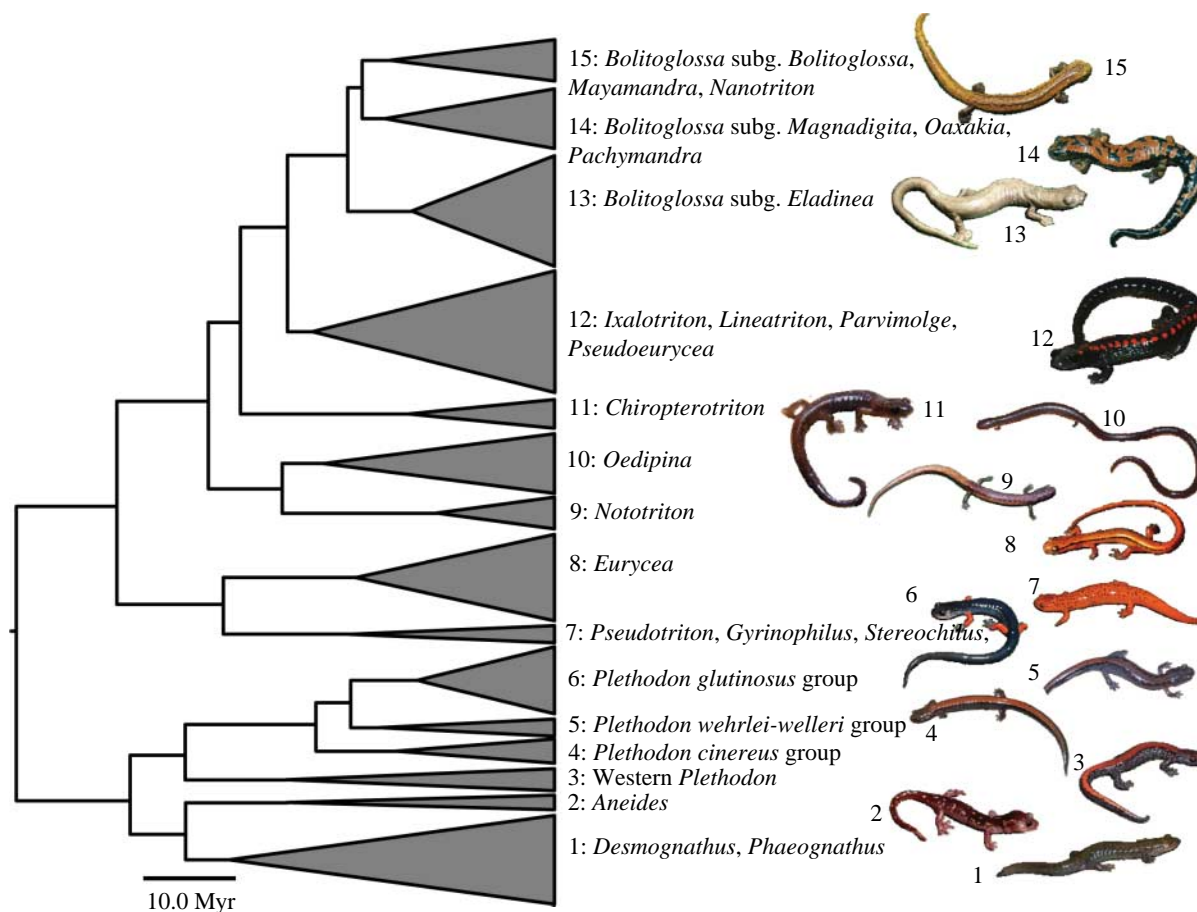
Figure 1. Reduced phylogeny of 15 clades of plethodontid salamanders analysed in this study (species-groups of *Plethodon* follow Wiens *et al.* 2006a and previous authors). Branch lengths reflect estimated ages of clades, whereas widths of each clade block are proportional to the total number of described species. The complete phylogeny of 190 species is provided in the electronic supplementary material. Images of representative species for each clade are shown (see acknowledgments for photo credits).

were estimated using all known species in each clade. However, another set of analyses used only species included in our phylogeny to estimate diversification rates, to assess whether biases were created by the reduced taxon sampling used to estimate rates of morphological evolution (i.e. based only on species included in the tree). Results were similar using different relative extinction rates and taxon sampling regimes (table 2s in the electronic supplementary material), and we present here only the results using all species and an intermediate relative extinction rate (0.45). We found no evidence that diversification rates are related to clade ages, so age seems unlikely to confound our comparison of rates among clades.

(d) *Morphological data*

We quantified morphological variation from 1573 adult plethodontid specimens from 260 species in 26 genera. Of these, 1262 specimens from 190 species could be matched to our phylogeny (appendices 3 and 4 in the electronic supplementary material). These data represent a combination of previously published measurements for 109 species (Wiens & Hoverman 2008) and new data for 81 species. For each specimen, we measured seven standard morphometric variables to the nearest 0.1 mm using digital calipers: snout–vent length (SVL), tail length (posterior end of cloaca to outstretched tail tip), body width (just posterior to forelimb insertion), head length (tip of snout to posterior corner of jaw), snout–eye distance (tip of snout to posterior corner of eye), forelimb length

(posterior insertion of forelimb to outstretched tip of longest finger) and hindlimb length (posterior insertion of hindlimb to outstretched tip of longest toe). Prior to combining the two datasets, a sample of specimens from the original dataset (Wiens & Hoverman 2008) were re-measured, and no evidence of inter-researcher bias was detected ($r > 0.988$ between measurements for all traits). Sample sizes for each species ranged from 1 to 24 individuals (mean = 6.7). Specimens of both sexes were combined, given that sexual-size dimorphism in plethodontids is seemingly minor relative to variation in adult body size within species (Petranka 1998). Given that sexual maturity can be difficult to ascertain without dissection, individuals were classified as adults if their SVL or total length was within the published range of adult sizes for that species and/or was similar to the largest individuals examined within that species (for species with many individuals available).

We performed a principal components analysis on the covariance matrix of the log-transformed measurements in R v. 2.80 (R Development Core Team 2008), and obtained PC scores for each individual and mean PC scores for each species. Variable loadings on PC1 were positive for all variables and were of similar magnitude. Thus, scores on PC1 were treated as an overall size measure (Jolicoeur 1963). Variable loadings on PC2–PC7 described relative differences in body proportions (tables 5s and 6s in the electronic supplementary material). Therefore, scores on axes PC2–PC7 were treated as estimates of body shape. We also plotted species' scores on PC1 and PC2 (which together

explain more than 92% of the morphological variation) to visualize placement of species and clades in morphospace. Because PC3–PC7 explained little of the total variation (less than 4% each), biological interpretations were based primarily on PC1 and PC2 (although we acknowledge that variation on other PCs could still be ecologically and evolutionarily important, even if they contribute little to overall morphological variation). Although PC2 may not be a 'pure' measure of shape (i.e. size may influence shape), examination of variable loadings for PC2 (table 5s in the electronic supplementary material) strongly suggest that it reflects elongation of the body and tail, and reduction in the length of the limbs, and as such describes variation in shape rather than size.

The seven measurements describe variation in body size and relative proportions of head, trunk, limbs and tail. Although many aspects of the functional ecomorphology of plethodontids are poorly studied, we anticipate that the relative sizes of the limbs, trunk and tail are important in locomotion and microhabitat usage, as in other vertebrates. For example, body elongation in plethodontid salamanders seems to be associated with fossoriality (e.g. Wake 1966; Jockusch 1997). Variation in head size is important in diet, given that plethodontids are generalist insectivores in which head or gape size determines prey size (e.g. Kryzsik 1979).

(e) *Rates of morphological evolution and morphological disparity*

Prior to estimating rates of morphological evolution, we compared the fit of two models of trait evolution to each clade. We used the OUCH package in R to calculate the likelihood of observing the pattern of morphological divergence in each clade under Brownian motion (BM) and Ornstein–Uhlenbeck (OU) models of evolution (Butler & King 2004). We then compared the fit of each model to the data using likelihood-ratio tests and the Akaike Information Criterion. We found that the BM model provided the best fit to patterns of size and shape evolution in all but two of the 15 clades (table 7s in the electronic supplementary material). Furthermore, rates of size and shape evolution estimated under BM and OU were very similar in the two clades for which the OU model was favoured (*Eurycea* and *Nototriton*).

Given the strong overall fit of BM across clades, we estimated rates of morphological evolution within clades using the BM model (following eqn (2) of O'Meara *et al.* 2006). The rate of morphological evolution ($\hat{\sigma}^2$), estimated from the set of measured traits and the phylogenetic covariance matrix, represents a time-independent measure of morphological change. For each clade, the maximum-likelihood estimator of $\hat{\sigma}^2$ was obtained separately for both body size and body shape. Because body shape was described by multivariate data (PC2–PC7), $\hat{\sigma}^2$ was a matrix containing evolutionary rates and rate covariances between traits (PC2–PC7). Thus, an estimate of the overall rate of body-shape evolution was estimated as the sum of the diagonal elements (the trace) of this rate matrix (see also McPeek *et al.* 2008). These rates were calculated in R v. 2.80 (R Development Core Team 2008).

To compare phylogenetic estimates of rates to non-phylogenetic estimates of disparity (e.g. Ricklefs 2004), we also calculated the total morphological disparity for each clade. Morphological disparity is the multivariate variance in morphospace among species, including all seven morphometric variables (following Foote 1993). Morphological disparity based on only shape (i.e. PC2–PC7) was also calculated.

(f) *Hypothesis testing*

For each clade, we calculated rates of (i) species diversification, (ii) morphological evolution in body size (PC1) and (iii) morphological evolution in body shape (PC2–PC7), as described above. We then tested the null hypothesis that rates of species diversification were positively related to rates of morphological evolution using *F*-ratios from regression (a one-tailed test). We took this as the null hypothesis given the previous empirical and theoretical work on diversity and disparity by Ricklefs (2004, 2006a) and Purvis (2004). To determine whether our data conformed to the assumptions of parametric models, we tested for multivariate normality (using a Shapiro–Wilks test) and homoscedasticity (using a Breusch-Pagan test). In all cases, homoscedastic variation was observed, and in only one case (species diversification versus size evolution) was normality violated. In this case, we ran a non-parametric analysis (Kendall's tau) to test the association between the two variables. Because clades are not independent (due to their phylogenetic relatedness), we also conducted analyses using phylogenetic generalized least squares (PGLS; Martins & Hansen 1997), using the topology and branch lengths from the chronogram described above. These analyses (and all statistical analyses described below) were conducted in R v. 2.80 (R Development Core Team 2008).

We examined the relationships among several additional clade-level parameters using regression. We tested for a relationship between morphological disparity and rates of species diversification, size evolution and shape evolution, and between disparity and clade age (following Ricklefs 2004). We also examined whether rates of size evolution were associated with rates of shape evolution among clades. To better understand the relationship between size and shape variation both within and between clades, we used mean species values for size (PC1) and shape (PC2), and tested for this relationship across all 190 species using PGLS and the full chronogram, assuming a BM model. Given that PC2 explains most shape variation (see above), we used PC2 as our primary estimate of shape for these analyses. Analyses using the OU model gave similar results (i.e. strong relationship between size and shape among species).

We also compared the 190 species included in our phylogeny to the other 70 species by visually examining the overlap between the two groups in plots of PC1 and PC2. This provided a qualitative assessment of whether variation among the species in our phylogeny was representative for each clade.

Finally, we quantified pairwise morphological overlap among clades in PC space (Butler *et al.* 2007). We divided the axes of morphospace (PC1 and PC2) into distinct regions, forming a grid that spanned the data space. The number of grid cells occupied by specimens from pairs of clades was then observed, and was treated as a quantitative measure of the overlap between clades. These patterns were statistically evaluated using a permutation procedure (Butler *et al.* 2007). To evaluate the robustness of our results to different grid-cell sizes, we repeated the overlap analysis with different numbers of grid cells (table 8s).

3. RESULTS

Estimated rates of species diversification and morphological evolution, along with related variables (crown-group age, diversity and disparity), are summarized in table 1.

Table 1. Summary data on 15 major clades of plethodontid salamanders (see figure 1). (Age is in millions of years ago (Myr ago) and pertains to the crown-group of the clade.)

clade	species in clade/ species included in tree	age	crown-group diversifi- cation rate (Myr; extinction=0.45)	size rate	shape rate	disparity
(1) *Desmognathus* and *Phaeognathus*	37/12	36.9	0.07343	0.03865	0.00072	1.04483
(2) *Aneides*	6/5	30.4	0.03220	0.01056	0.00027	0.33346
(3) Western *Plethodon*	9/6	30.5	0.04435	0.00423	0.00027	0.10406
(4) *P. cinereus* group	10/7	18.1	0.08047	0.00615	0.00061	0.12927
(5) *P. wehrlei-welleri* group	7/6	19.9	0.05621	0.01385	0.00025	0.49880
(6) *P. glutinosus* group	28/18	15.7	0.15849	0.01679	0.00062	0.20600
(7) *Gyrinophilus, Pseudotriton* and *Stereochilus*	7/4	23.4	0.03487	0.01565	0.00036	0.50082
(8) *Eurycea*	36/17	22.7	0.10472	0.09287	0.00205	0.94043
(9) *Nototriton*	13/5	13.5	0.12685	0.01509	0.00172	0.30909
(10) *Oedipina*	25/10	18.0	0.12946	0.02155	0.00157	0.46246
(11) *Chiropterotriton*	12/7	16.6	0.09760	0.04890	0.00043	0.73820
(12) *Pseudoeurycea* clade	51/32	27.6	0.10717	0.05166	0.00231	0.77938
(13) *Bolitoglossa*, subgenus *Eladinea*	46/15	16.3	0.17988	0.02225	0.00072	0.31299
(14) *Bolitoglossa*, subgenera *Magnadigita, Oaxakia* and *Pachymandra*	25/19	19.4	0.12069	0.02981	0.00079	0.48221
(15) *Bolitoglossa*, subgenera *Bolitoglossa, Mayamandra* and *Nanotriton*	17/10	18.8	0.10435	0.01832	0.00083	0.49546

There was no significant relationship between rates of species diversification and rates of morphological evolution, for either size or shape (figure 2a,b; table 2), nor between rates of species diversification and morphological disparity (figure 2c). Similarly, for species diversification versus size evolution, non-parametric analyses showed no association (Kendall's tau=0.2762; $p=0.1513$). Clade age predicted patterns of morphological disparity (table 2; Purvis 2004; Ricklefs 2004, 2006a), but did not predict species diversification rates nor rates of morphological evolution (results not shown). Morphological disparity was significantly associated with rates of size evolution (figure 2e), but not shape evolution (table 2). However, shape disparity was associated with rates of shape evolution. There was a significant positive relationship between rates of evolution in size and shape among clades (figure 2d). Additionally, size and shape were significantly associated when examined across species within and between clades (PGLS: $\beta=-0.114$; $T=-5.25$; $p<0.0001$). The patterns above were robust to (i) incorporating phylogeny with PGLS (table 2), (ii) different relative extinction rates, (iii) excluding taxa in estimates of diversification rates and (iv) alternative clade divisions (tables 1s, 2s, 3s and 4s in the electronic supplementary material).

There was considerable overlap in morphospace among many of the 15 clades (figure 3a), at least for PC1 and PC2, which together account for 92.2 per cent of the morphological variance (table 6s and fig. 1s in the electronic supplementary material). Pairwise overlap measures largely confirmed these findings (table 8s in the electronic supplementary material). Most clades had similar body shapes and many even showed similar ranges of body sizes (figure 3b; see also Kozak *et al.* in press for North American clades). However, some clades

(e.g. *Nototriton, Oedipina* and *Eurycea*) exhibited little overlap with other clades, implying more distinct morphologies (table 8s in the electronic supplementary material). *Oedipina* and *Lineatriton* were particularly divergent in body shape (PC2), having elongate tails and short hindlimbs (figure 3a). Finally, there was nearly complete overlap in morphospace between species included in the phylogeny and those not included, implying that selection of taxa for inclusion in the phylogeny and subsequent analyses did not bias interpretation of morphological patterns (results not shown).

4. DISCUSSION

A general relationship between rates of diversification and rates of morphological evolution may be expected for several reasons. For example, the ecological theory of adaptive radiation predicts accelerated rates of speciation associated with divergence in ecologically relevant phenotypic traits (e.g. Schluter 2000), at least at some points in the radiation process. The hypothesis of punctuated equilibrium (e.g. Gould & Eldredge 1977) predicts that most evolutionary change occurs at speciation events, which might also lead to a correlation between rates of species diversification and morphological change (e.g. Ricklefs 2006a; Bokma 2008), in some cases (e.g. if diversification rates vary among clades). A previous study on species diversity and morphological disparity in birds (Ricklefs 2004) also implied that these rates were correlated. However, simulations suggested that morphological variance and species richness of clades should generally be correlated (Purvis 2004; Ricklefs 2006a), due to the dependence of both variables on clade age (i.e. diversity and disparity should be high in old clades, even if rates of species diversification and morphological evolution are similar across clades).
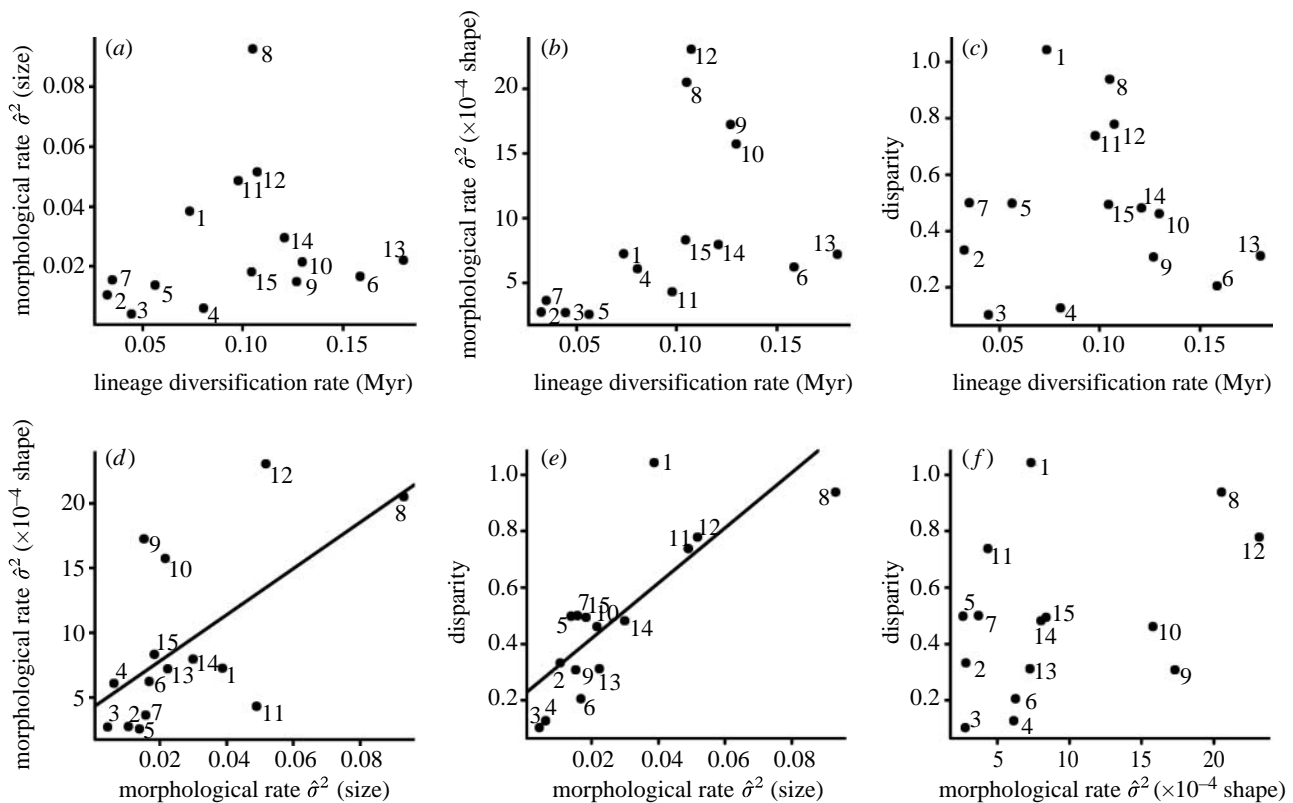
Figure 2. Relationships between rates of morphological size evolution, morphological shape evolution and species diversification among 15 clades of plethodontid salamanders (clade numbers follow table 1). (*a*) Diversification rate versus rate of size evolution; (*b*) diversification rate versus rate of shape evolution; (*c*) diversification rate versus morphological disparity; (*d*) rate of size versus shape evolution; (*e*) rate of size evolution versus morphological disparity; and (*f*) rate of shape evolution versus morphological disparity. Only significant regressions are displayed. Full results are provided in table 2.

Despite these expectations, our results show that rates of morphological diversification and species diversification are not significantly correlated in plethodontid salamanders. For example, the clade with the highest rate of species diversification (*Bolitoglossa* subgenus *Eladinea*) has unexceptional rates of morphological evolution, and the clade with the highest rate of change in size and second highest rate of shape evolution (*Eurycea*) has only a moderate rate of species diversification (table 1). Our results also suggest that morphological disparity may not always be an adequate proxy for rates of change, as we found a relationship between standard measures of disparity and rates of size change, but not shape change, in plethodontids (though rates of shape change are related to shape disparity). This result reinforces the need to examine rates of phenotypic change and species diversification in a phylogenetic context.

In theory, the lack of a significant relationship between rates of species diversification and morphological evolution might be explained by one of several methodological artefacts, but this seems unlikely. First, this correlation might exist, but the power of our study may be too weak to detect it (e.g. too few clades, not enough variation among them). However, rates of size and shape evolution were sufficiently variable to show significant associations with other variables (i.e. disparity, each other), and rates of species diversification also show significant variation among clades (see below). Second, our sampling of species within clades, although extensive (190), is not complete. However, our estimates of species diversification rates do incorporate all described

species within each clade, regardless of whether they are included in our phylogenies. Incomplete sampling could affect estimates of morphological rates, but we also find no significant relationship between species diversification and morphological evolution when we estimate both rates only from species included in the phylogeny (table 2s in the electronic supplementary material). Additionally, the broad morphological overlap between taxa included in our phylogeny and those not included suggests that patterns of morphological variation we observed were not biased by excluding these taxa. Our results are also robust to alternative divisions of plethodontids into clades (electronic supplementary material). We acknowledge that our conclusions about morphological evolution are confined to those characters that we measured; namely body size and the relative proportions of head, trunk, limbs and tail. Although these characters describe dramatic variation in body size and shape, other traits are not included, such as modes of tongue projection (e.g. Lombard & Wake 1986), cranial morphology (e.g. Wake 1966; Adams 2004; Adams *et al.* 2007) and foot morphology (e.g. Alberch 1981; Jaekel & Wake 2007). However, even if we have neglected to include a 'key innovation' trait that explains variation in diversification rates between clades, our goal here is to determine whether clades with accelerated diversification rates also have accelerated rates of within-clade morphological evolution. Although morphologically cryptic species have been documented in most plethodontid clades (and represent obvious decoupling of species diversification and morphological change), these do not seem to explain the pattern either. For example, many

Table 2. Statistical summary of linear regressions between rates for the seven major clades described in table 1. (The first set of analyses (tips) do not take phylogeny into consideration, while the second set of analyses (PGLS) account for the phylogenetic relationships among clades.)

| analysis	analysis	β	s.e.	T	p-value
diversification rate versus size rate	tips	0.105	0.144	0.729	0.479
diversification rate versus size rate	PGLS	0.086	0.118	0.733	0.477
diversification rate versus shape rate	tips	0.007	0.004	1.707	0.112
diversification rate versus shape rate	PGLS	0.003	0.004	0.793	0.442
diversification rate versus disparity	tips	-0.363	1.782	-0.204	0.842
diversification rate versus disparity	PGLS	-1.448	1.585	-0.908	0.381
size rate versus shape rate	*tips*	*0.018*	*0.0006*	*2.796*	*0.015**
size rate versus shape rate	*PGLS*	*0.020*	*0.007*	*2.851*	*0.014**
size rate versus disparity	*tips*	*9.818*	*1.992*	*4.928*	*0.0003**
size rate versus disparity	*PGLS*	*9.737*	*2.638*	*3.691*	*0.003**
shape rate versus disparity	tips	170.617	105.218	1.622	0.129
shape rate versus disparity	PGLS	161.331	110.359	1.462	0.168
shape rate versus shape disparity	*tips*	*44.45*	*15.32*	*2.90*	*0.0124**
shape rate versus shape disparity	*PGLS*	*37.27*	*16.09*	*2.32*	*0.0375**
age versus disparity	tips	0.016	0.011	1.504	0.156
age versus disparity	*PGLS*	*0.031*	*0.012*	*2.595*	*0.022**
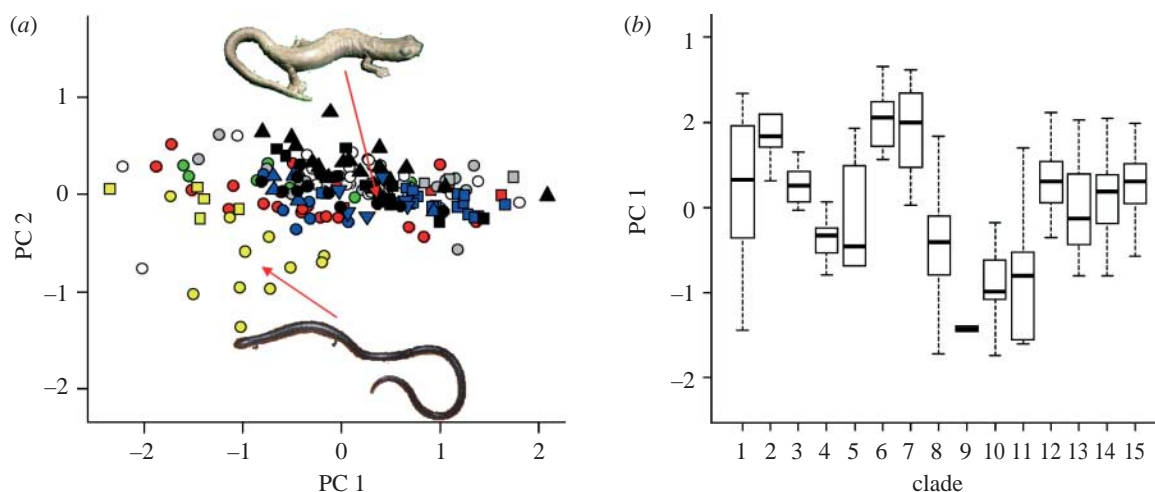
Figure 3. (*a*) Principal component plot of the morphometric data. Mean size and shape scores for each species are displayed. PC1 (size) describes 80.8% of the total variation, whereas PC2 (shape) explains 11.4% of the total variation. Species from major plethodontid clades are shown in similar colours; symbol and colour combinations signify 15 clades examined in this study. (*b*) Box and whiskers plot of PC1 for each of the 15 clades (medians and quartiles shown). Clades are: 1: *Desmognathus*, *Phaeognathus* (grey circle); 2: *Aneides* (grey square); 3: Western *Plethodon* (blue down triangle); 4: *Plethodon cinereus* group (blue circle); 5: *Plethodon wehrlei-welleri* clade (blue up triangle); 6: *Plethodon glutinosus* group (blue square); 7: *Pseudotriton*, *Gyrinophilus*, *Stereochilus* (Spelerpinae; red square); 8: *Eurycea* (Spelerpinae; red circle); 9: *Nototriton* (yellow square); 10: *Oedipina* (yellow circle); 11: *Chiropterotriton* (green circle); 12: *Pseudoeurycea* clade (*Ixalotriton*, *Lineatriton*, *Parvimolge*, *Pseudoeurycea*; white circle); 13: *Bolitoglossa* (subgenus *Eladinea*; black circle); 14: *Bolitoglossa* clade (subgenera *Magnadigita*, *Oaxakia*, and *Pachymandra*; black up triangle); 15: *Bolitoglossa* (subgenera *Bolitoglossa*, *Mayamandra*, and *Nanotriton*; black square). In (*a*), two species are shown to demonstrate typical body proportions of taxa found in different regions of the plot (arrows indicate placement of each species): above, *Bolitoglossa colonnea* from *Bolitoglossa* (subgenus *Eladinea*); below, *Oedipina gracilis*, representing *Oedipina*.

morphologically cryptic species are known in *Eurycea* (Kozak *et al.* 2006*b*), yet this genus has high rates of morphological evolution but not species diversification (table 1). If cryptic species explained the lack of relationship, one would expect high rates of species diversification accompanied by low rates of morphological change.

Given our finding of no significant relationship between species diversification and morphological evolution, what might explain patterns of variation in these variables? Some variation in species diversification rates may be explained by the latitudinal position of clades. A relationship between low latitudes and high diversification rates has been found in many previous studies, including salamanders and

frogs (Wiens 2007), birds (Cardillo 1999; Cardillo *et al.* 2005; Ricklefs 2006*b*), butterflies (Cardillo 1999) and palms (Svenning *et al.* 2008). In plethodontids, rates of diversification are significantly higher in tropical clades (Bolitoglossinae, clades 9–15) than temperate clades (Pletho-dontinae, clades 1–6; Spelerpinae, clades 7–8), based on a *t*-test of data in table 1 (d.f. = 13; *p* = 0.02; but note that the tropical clades are all closely related). The tropical bolito-glossines are the only primarily tropical clade of salamanders and collectively have the highest species diversification rate of any major salamander clade (Wiens 2007). This pattern may be related to higher extinction rates in temperate clades or higher speciation rates in tropical clades. Regardless of

the rate of diversification, speciation in tropical salamanders seems to have a strong climatic component; sister species tend to occur in different climatic environments associated with different elevations, whereas temperate sister species tend to occur in similar environments (Kozak & Wiens 2007). If speciation occurs primarily through geographic isolation related to climatic factors, there may be little reason to expect morphological divergence to generally accompany speciation (unless climate-related factors impose selection on body form). Similarly, reproductive isolation may be promoted by divergence in courtship pheromones (e.g. Palmer *et al.* 2005, 2007), which may not lead to morphological differences among species.

One might expect higher species diversification rates to be related to the invasion of morphospace not occupied by other plethodontid clades, given the idea that a clade will tend to constrain the diversification of sympatric, phenotypically similar clades (e.g. Schluter 2000). However, this prediction is not supported in plethodontids (see also Kozak *et al.* in press). In the traits we examined, most plethodontid clades show surprisingly similar patterns of morphological variation; most clades exhibit similar body shape (PC2) and even a similar range of body sizes (PC1), and differences between species within clades seem to mostly involve sliding up and down this range of body sizes (figure 3b). Only a few clades have deviated from this pattern. The most obvious exception is *Oedipina* (figure 3a), which shows little overlap with other clades in morphological space (due to an elongate tail and short limbs). However, despite its unusual morphology, *Oedipina* shows only moderate rates of species diversification (table 1). The *Pseudoeurycea* clade has also invaded a portion of morphospace similar to *Oedipina*, and has high rates of species diversification, size change and shape change. However, only three species of the *Pseudoeurycea* clade have this worm-like morphology (i.e. the three species of *Lineatriton*), and most species diversification has instead involved species with more typical body forms (figure 3a). In summary, most species diversification in plethodontids has occurred in a relatively small region of morphospace, a region that is shared by most clades (figure 3a). Similarly, Ricklefs (2005) found that clades of passerine birds with limited species richness tended to be in the periphery of multivariate morphospace, relative to more diverse clades.

Patterns of species diversification also appear generally unrelated to allopatry of clades, with one important exception. Notably, the highest rates of species diversification in plethodontids are in the subgenus *Eladinea* of *Bolitoglossa* (table 1), which occurs primarily in South America and is mostly allopatric with respect to other plethodontid clades (Wiens *et al.* 2007). However, other plethodontid clades are sympatric with one or more other clades, despite considerable variation in diversification rates. For example, the *Plethodon glutinosus* group has a diversification rate approaching that of *Eladinea* (table 1), but is microsympatric with up to five other plethodontid clades in many localities in eastern North America (clades 1, 4, 5, 7, 8; Petranka 1998). There is also extensive sympatry between clades of tropical bolitoglossines (e.g. *Chiropterotriton* and *Pseudoeurycea* in Mexico; *Bolitoglossa*, *Nototriton* and *Oedipina* in Central America; Wake 1987). Intriguingly, we see no obvious

shifts in morphospace associated with either allopatry or sympatry of these clades (figure 3a).

We also found that rates of size and shape evolution are strongly correlated. To our knowledge no previous studies have tested for this relationship. Why should these variables be correlated? One hypothesis is that if ecomorphs differ in both size and shape (e.g. West Indian *Anolis* lizards; Losos *et al.* 1998), then rates of size and shape evolution will be coupled and will depend on the rate at which ecomorphs evolve in each clade. Shared allometric relationships among species within clades (i.e. similar shapes evolve when similar sizes evolve) might also cause these rates to be correlated. Alternately, within a given clade there could be rapid size evolution in one subclade and rapid shape evolution in another; this could lead to high rates of evolution in both traits among clades, even without a correlation within clades. However, we also found a general relationship between size and shape evolution at the species level, which parallels the correlated patterns of rate evolution among clades. Although much morphological evolution in plethodontids seems to involve clades with similar body shapes undergoing shifts in body size, at least in some clades, shifts in body size are also accompanied by changes in body shape.

In this study, we present the first direct test of whether rates of species diversification and morphological evolution are correlated across clades, and find no significant relationship, despite theoretical and empirical expectations. The generality of these results will need to be tested in other groups of organisms, preferably using similar phylogeny-based approaches. Our results imply that speciation in plethodontids typically is not accompanied by extensive phenotypic divergence, but may be associated instead with other factors that bear more directly on reproductively isolating populations, such as climatic factors limiting geographic ranges. Our results also suggest that patterns of morphological evolution in body form are highly redundant across plethodontid clades, with most clades sharing similar general body form and diverging primarily in body size, regardless of their rates of species diversification or the presence of other sympatric clades.

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