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Ecological radiation with limited morphological diversification in salamanders

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Abstract

A major goal of evolutionary biology is to explain morphological diversity among species. Many studies suggest that much morphological variation is explained by adaptation to different microhabitats. Here, we test whether morphology and microhabitat use are related in plethodontid salamanders, which contain the majority of salamander species, and have radiated into a striking diversity of microhabitats. We obtained microhabitat data for 189 species that also had both morphometric and phylogenetic data. We then tested for associations between morphology and microhabitat categories using phylogenetic comparative methods. Associations between morphology and ecology in plethodontids are largely confined to a single clade within one subfamily (Bolitoglossinae), whereas variation in morphology across other plethodontids is unrelated to microhabitat categories. These results demonstrate that ecological radiation and morphological evolution can be largely decoupled in a major clade. The results also offer a striking contrast to lizards, which typically show close relationships between morphology and microhabitat.

Introduction

A major goal of evolutionary biology is to explain the morphological diversity of plants and animals. In animals, many studies suggest that adaptation to different microhabitats is a major factor explaining morphological differences among species within a clade (e.g. Wainwright & Reilly, 1994), especially in the case of adaptive radiations (e.g. Grant, 1999; Schluter, 2000; Clabaut *et al.*, 2007; Gavrilets & Losos, 2009). However, a few studies have also found morphological variation to be uncorrelated with microhabitat usage, but typically in smaller clades occupying a limited range of terrestrial microhabitats (e.g. Vanhooydonck & Van Damme, 1999; Schulte *et al.*, 2004).

Here, we test whether morphology and microhabitat usage are related in plethodontid salamanders. Plethodontidae contains the majority of salamander species (416 of 608 species; AmphibiaWeb, 2011). Plethodontids have undergone an impressive ecological radiation into different microhabitats, and utilize nearly the full range of microhabitats used by nonmarine and nonvolant vertebrates, including species that are fossorial, arboreal, terrestrial, saxicolous, and fully aquatic (e.g. Wake, 1987; Petranka, 1998).

Many studies have examined the evolutionary morphology and diversification of plethodontids (e.g. Lombard & Wake, 1986; Jockusch, 1997; Adams & Rohlf, 2000; Adams *et al.*, 2009; Kozak *et al.*, 2009). However, few studies have addressed the relationship between microhabitat and morphology, and these studies have generally been confined to single genera (e.g. *Desmognathus*; Kozak *et al.*, 2005; *Bolitoglossa*; Jaekel & Wake, 2007). Although data on microhabitat use and morphology are present in the literature for many plethodontid species, little is known about family-wide relationships between morphology and microhabitat (Wake, 2009).

Plethodontids are also an intriguing group for studies addressing ecomorphological relationships because they

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(and salamanders in general) are similar in body form to lizards but only distantly related to them. Like plethodontids, lizards have radiated into a remarkable diversity of microhabitats (Vitt & Caldwel, 2009). The relationship between morphology and microhabitat usage in lizards is relatively well studied, and much of the variation in lizard morphology (especially the size and shape of the head, limbs, and tail) is attributable to microhabitat use in many groups (e.g. Losos, 1990a; Irschick *et al.*, 1997; Vitt *et al.*, 1997; Wiens *et al.*, 2006; but see Vanhooydonck & Van Damme, 1999; Schulte *et al.*, 2004). Despite the similarities in body form, there are also major differences between lizards and plethodontid salamanders in physiology, energetic costs of locomotion, and behaviour (e.g. plethodontids prefer low temperatures, have low metabolism, and are lungless; Vitt & Caldwel, 2009).

In this study, we test the relationships between morphology and microhabitat usage in plethodontid salamanders. We obtain microhabitat data from the literature for 189 species for which both morphological and phylogenetic data are available (Adams *et al.*, 2009). We then test whether microhabitat is related to morphological variation using phylogenetic comparative methods. Remarkably, we find that the impressive ecological radiation of plethodontids is largely decoupled from variation in morphology (with the exception of a single clade, the tropical bolitoglossines). These results suggest that plethodontid salamanders, unlike lizards, generally retain similar morphologies across a wide range of microhabitats.

Materials and methods

Phylogeny

The time-calibrated phylogeny used in this study was taken from Adams *et al.* (2009). This phylogeny was constructed by combining a phylogeny for 109 plethodontid species based on nuclear and mitochondrial DNA data (including all genera and most species in North America, from Kozak *et al.*, 2009), and a phylogeny for 137 species of tropical bolitoglossines (including all genera and about a third of the species) from Wiens *et al.* (2007), based on mitochondrial DNA data. This combined phylogeny was then reduced to include only those species for which morphological data were available (see below). Most analyses are based on the phylogeny with a root age of 61 Myr (million years). However, we also tested the robustness of our results to trees with both younger and older root ages for Plethodontidae (48 and 69 Myr; Adams *et al.*, 2009; note that these different root ages can influence the relative lengths of branches, not just the overall depth of the tree). We note that although different studies have inferred various root ages for Plethodontidae, the ages of many major clades within Plethodontidae are similar in our study and a recent multi-locus analysis using Bayesian divergence-time methods across salamanders (Zheng *et al.*, 2011). For example, estimates for Spelerpinae, *Desmognathus–Phaeognathus*, *Aneides*, and *Plethodon* are within ~5 Myr or less of each other.

Morphology

Morphological data for seven variables for 189 species were obtained from Adams *et al.* (2009). These data are derived from 1296 museum specimens, including data from 108 species from Wiens & Hoverman (2008) and from 81 additional species from Adams *et al.* (2009). The sample size varied between 1 and 24 specimens per species (only adults included), with a mean of 6.7 individuals per species. The morphological variables are the species means for: snout–vent length (SVL), tail length (TL; the posterior end of the cloaca to the tail tip), head length (HL; tip of the snout to the posterior corner of the jaw opening), body width (BW; measured just posterior to the forelimb insertion), snout–eye distance (SE; tip of the snout to posterior corner of the eye opening), and forelimb and hindlimb length (FLL and HLL; the posterior insertion of the limb to the tip of the longest digit, with limbs outstretched). Sexual-size dimorphism in plethodontids appears to be minor relative to variation in adult body size within species (e.g. Petranka, 1998), and so data from both sexes were combined. Prior to all statistical analyses, the variables were log-transformed to better meet the requirements of normality and homogeneity.

To remove the effect of size from these linear measurements, Burnaby's method (Burnaby, 1966) was applied, following Adams & Rohlf (2000). The species-morphology matrix containing the log-transformed morphological data was projected on the isometric size vector and returned to the original coordinate system according to the equation:

$$X(I - \mu(\mu\mu')^{-1}\mu'),$$

where X is the data matrix, I is the identity matrix, and μ represents the isometric growth vector (Burnaby, 1966; McCoy *et al.*, 2006). The size measure that accompanies these shape variables is the geometric mean of the linear measurements for each species (see Mosimann, 1970). This approach to size and shape is generally preferable over regression-based methods, which can generate patterns of false allometry (Mosimann, 1970; Mosimann & James, 1979). Furthermore, Burnaby's method is preferable in this particular case because plethodontid salamanders are characterized by a high degree of ontogenetic stasis (i.e. isometry; Jaekel & Wake, 2007; Adams & Nistri, 2010).

For comparison, the phylogenetic comparative analyses described below were also performed on the nonsize-adjusted data. By performing a PCA (see below) the effect of size was captured by PC1, while the remaining PCs

represent different relative proportions of the morphological variables (i.e. shape). All statistical analyses were performed in R version 2.12.1, and, hereafter, only the specific packages used will be referred to. The R codes for all analyses are provided in Appendix S1.

Microhabitat

To test the relationship between body form and microhabitat use, species were assigned to microhabitat categories using data from the literature. Microhabitat data were obtained from various sources, including Wake (1987), Petranka (1998), McCranie & Wilson (2002), AmphibiaWeb (2011), and IUCN (2010). The microhabitat categories we primarily used were: arboreal (A), aquatic (W), semi-aquatic (SW), bromeliad (B), fossorial (F), saxicolous (S), moss mats (M), and terrestrial (T). Microhabitat assignment for each species is shown in Table S1.

The boundaries of each category are explained below, along with alternative categorizations. Species were considered arboreal (A) if they typically climb in vegetation higher than 1 m above the ground and are encountered on leaves, branches, trunks, or epiphytes. The importance of bromeliad epiphytes (B) for the ecology of salamanders is sometimes emphasized (e.g. Wake, 1987). Therefore, we performed some analyses in which species frequenting bromeliad epiphytes were assigned to a microhabitat category separate from the other arboreal species. Moss mats (M) have also been emphasized as a distinct microhabitat type (e.g. Wake, 1987). Therefore, species that are frequently encountered on terrestrial moss mats or dense moss on tree trunks were assigned to the moss mats category in one analysis. Saxicolous (S) species are those that live in or on rocky substrates (i.e. within caves or in their twilight zones, on rock outcrops, or on rocky boulders). Species that generally live on nonrocky substrates but are sometimes found under rocks were not categorized as saxicolous. Species were considered fossorial (F) if they live in burrows and are rarely encountered outside them. Species that spend their entire adult life underwater (e.g. paedomorphic, nonmetamorphosing species) were assigned to the aquatic (W) microhabitat category. Semi-aquatic (SW) microhabitat use was assigned to species that, during adulthood, routinely spend long periods under water (e.g. for mating), but also spend considerable time on land. Species that merely live along stream edges or in splash zones of waterfalls (without submerging in the water for long periods of time) were not assigned to this category. Finally, terrestrial species include those that are active primarily on the ground, including those that spend much of their time under rocks, logs, debris, or vegetation.

Given that there are potentially many reasonable ways to assign species to microhabitats, we examined several alternate coding strategies (scenarios; see descriptions in Table 1). These scenarios also account for within-species variation in microhabitat use (Table S1). Species that occur both in arboreal and bromeliad microhabitats are assigned to the former category in scenario 1 and to the latter in scenario 5. Semi-aquatic species are assigned to the terrestrial category in scenario 2 and to the aquatic category in scenario 3. Also, fossorial species are treated separately in the third scenario. In scenario 4 all climbing species (arboreal, bromeliad, and saxicolous categories) are combined into one category. Species that were recorded in terrestrial or arboreal moss mats are assigned

Table 1 Microhabitats included in the nine categorical scenarios.

Scenario	Microhabitats	Description
1	T, W, SW, A (incl. B), S (incl. F)	Tests effect of combining bromeliad with arboreal ecotypes, given that living in bromeliads requires climbing on vegetation
2	T (incl. SW), W, A (incl. B), S (incl. F)	Same as scenario 1, but tests including semi-aquatic species as terrestrial
3	T, F, W (incl. SW), A (incl. B), S	Same as scenario 2, but tests the effects of combining the semi-aquatic and aquatic categories
4	T (incl. F), W, SW, A+S+B	Tests importance of climbing, regardless of the substrate, by combining arboreal, bromeliad, and saxicolous ecotypes.
5	T, W, SW, A, B, S (incl. F)	Tests effect of the bromeliad ecotype on morphology separate from other ecotypes
6	T, W, SW, A (incl. B), S (incl. F), M	Tests the consequence of frequenting moss mats*
7	T, W, SW, A, B, S (incl. F)	Reassigns some species of the genus *Eurycea* to the saxicolous instead of the semi-aquatic category
8	T, W, SW, A (incl. B), S (incl. F)	Least possible assignments to terrestrial microhabitat: only terrestrial microhabitat specialists were categorized as terrestrial
9	T (incl. S, F, SW), W, A (incl. B)	Maximal possible assignments to terrestrial microhabitat: all species using terrestrial microhabitats were categorized as terrestrial

Microhabitat abbreviations are: A (arboreal), B (bromeliad), F (fossorial), S (saxicolous), SW (semi-aquatic), T (terrestrial), W (aquatic), and M (moss mats).

* All individuals that were assigned to the moss mat ecotype in scenario 6 are assigned to other microhabitat categories in the remaining five scenarios.

to a moss mat category in scenario 6, and are treated as either terrestrial or arboreal under other scenarios. The genus *Eurycea* contains many species that occur in saxicolous microhabitats in addition to aquatic microhabitats and these species are classified as saxicolous in scenario 7. In scenario 8, species that occur in both arboreal and terrestrial microhabitats are treated as arboreal and species that occur in both saxicolous and terrestrial microhabitats are categorized as saxicolous. In contrast, in scenario 9, these two sets of species are both categorized as terrestrial.

Taken together, these various coding strategies generally account for species that occur in two or more microhabitats (i.e. under one coding strategy, they are coded as occurring in one of their microhabitats, and are coded as occurring in another under an alternative strategy). Nevertheless, a 'default' microhabitat was still needed in some cases. This default microhabitat category was chosen as the microhabitat in which the species was most frequently encountered, based on the references cited (the predominant microhabitat is listed in the first column in Table S1). However, in a minority of cases, it was not clear which microhabitat was predominant, and these were coded somewhat arbitrarily. But again, even these cases are accounted for by our various coding strategies. Among the 189 species, there are three species that occur in multiple microhabitats (and in which some of the microhabitats used by the species are not included in the coding schemes), but in all three of these species one of the microhabitats is more commonly used than the others, making the coding relatively unambiguous.

We also performed a series of analyses in which we tested specific microhabitats against all other microhabitats (treating microhabitat as binary). These included: arboreal and bromeliad (scenario 10), terrestrial and semi-aquatic (scenario 11), aquatic and semi-aquatic (scenario 12), saxicolous and fossorial (scenario 13), bromeliads (scenario 14), and a combination of arboreal and saxicolous (scenario 15). For species occurring in multiple microhabitats, any occurrence in the microhabitat in question was coded as present.

Ecological radiation

In order to visualize the microhabitat variation among species on the tree, we used maximum likelihood reconstruction (e.g. Schluter *et al.*, 1997; Pagel, 1999). This was implemented in Mesquite version 2.74 (Maddison & Maddison, 2010), with the Markov k-state, one-rate model (a single rate for all transitions between all states, the most practical method given the large number of states). To better visualize the variation among species, we included all eight microhabitat categories as character states, except for the moss mats category. We consider moss mats as a secondary microhabitat, utilized by species in addition to either terrestrial or arboreal microhabitats.

Principal components analysis

After size-correction, species means for the morphological variables were included in a principal components analysis (PCA), using the pcaMethods package (Stacklies *et al.*, 2007). The PCA was performed on the covariance matrix and six axes were retained. Species scores along each PC axis were obtained and used in the phylogenetic comparative analyses. Similarly, a PCA was performed on the covariance matrix of the seven log-transformed (but not size-adjusted) morphological variables for comparison with the results of the analyses on the size-corrected variables.

Phylogenetic comparative analyses

To address the relationship between morphology and microhabitat, we used phylogenetic generalized least squares (PGLS; Grafen, 1989; Martins & Hansen, 1997). In PGLS, the original (nonphylogenetically corrected) data are converted by multiplication with the variance–covariance matrix derived from the tree (e.g. using the vcv.phylo function in the ape package; Paradis *et al.*, 2004). This can be done using different models of evolution, including Brownian motion (BM) and Ornstein–Uhlenbeck (OU).

The effects of size were represented by the geometric mean vector obtained after the Burnaby size-correction, and PC1–PC6 represented shape (Table 2). When no size-correction was applied prior to the PCA, PC1 was considered to represent size, since the loadings for all the morphological variables were roughly equal on this axis (Jolicoeur, 1963). The remaining six PCs show different patterns of variation in different sets of morphological variables, and are considered to represent variation in shape.

Using the size-corrected variables, we tested if body size and shape (size-vector and multivariate shape; dependent variables) are each related to microhabitat use (independent variables), using the 15 scenarios for categorizing microhabitat use (Table 1). For shape, we first examined the effect of microhabitat use on PC1–PC6 simultaneously by performing a multivariate PGLS for the microhabitat scenarios (using the nine categorical scenarios only). Presently, no R packages are available that perform multivariate PGLS, as the summary.manova routine does not retain covariance structures passed to it. Therefore we wrote a code for PGLS that handles a multivariate Y variable (see R-codes in Appendix S1). Estimates of lambda (phylogenetic signal) and r^2 were not obtained, as neither has been developed for multivariate models.

In addition, we performed an exploratory analysis using each of the six PC-axes separately as response variables in a PGLS with the forward selection scheme for the microhabitat scenarios. To find the best possible evolutionary model for the (univariate) PGLS, we first

used PGLS implemented in CAIC (Orme *et al.*, 2009) to obtain the maximum likelihood estimation of the strength of phylogenetic signal (λ; Pagel, 1999). We also used nlme (Pinheiro *et al.*, 2011) to find the expected variance-covariance matrix under a given evolutionary model (i.e. BM or OU). Using other PGLS models (e.g. from the caper package; Orme *et al.*, 2011) did not affect the results and are therefore not included in the analyses. The likelihood of the data under the BM and OU models for each PC was estimated using the geiger package (Harmon *et al.*, 2008). The actual covariance structure under each model was obtained in the PGLS analysis (see R-codes in Appendix S1). For each scenario, the most likely model was selected using the Akaike Information Criterion (Akaike, 1983). One model was preferred over another if the AIC scores differed by at least four (Burnham & Anderson, 2002). When AIC scores differed by < 4, we performed the analysis with both models. There was only one instance in which we found two models with AIC scores that differed by < 4 (see 'Results' section). Here, the models showed similar results and we present only the results of one of the two models in the 'Results' section.

For the size-uncorrected variables, we analysed the relationship between microhabitat use and morphology using the microhabitat classification schemes in scenarios 1–6. The first six scenarios together test the effects of all microhabitat categories included in our analysis (and gave results very similar to those for the size-corrected data; see 'Results' section) and were therefore considered sufficient for robustness analyses, rather than testing all possible scenarios. Similarly, we used the most likely model and the first six scenarios to test the effect of different root ages (i.e. 48 and 69 Myr) for the time-calibrated tree. The results were compared with the results obtained from the original analysis with size-corrected variables and a root age of 61 Myr.

Sequential Bonferroni correction was applied to the results following Rice (1989). This correction redefines the significance threshold as $P \leq \alpha/(1 + k - i)$, for the ith P-value of k tests. Only P-values that remained below the significance threshold after sequential Bonferroni correction were considered significant.

Given that ecomorphological associations may differ between clades and regions, temperate and tropical plethodontids were analysed separately in some analyses. We assigned all tropical bolitoglossines (106 species; including all tropical genera) to a tropical subgroup. The remaining 83 species were assigned to a temperate subgroup (including one genus of Bolitoglossinae, one of Hemidactyliinae, four of Spelerpinae, and seven of Plethodontinae). Note that the tropical bolitoglossines are a monophyletic group whereas the temperate subgroup is not, but this should not adversely influence the analysis. The PCA and PGLS analyses described above were performed on these subgroups separately. However, fewer scenarios were used in these PGLS analyses, as not all microhabitats were represented in both subgroups. For the temperate subgroup we analysed scenarios 1–4, 6, 10–13, and 15. For the tropical subgroup the analysis was restricted to scenarios 1, 4, 5, 10, 13, and 15. For both groups we considered the effects of microhabitat partitioning on both shape (i.e. PC1–PC6 in the case of Burnaby size-corrected variables and PC2–PC7 in the case of size-uncorrected variables) and size (i.e. the geometric mean derived from the Burnaby size-correction and PC1 for the size-uncorrected data).

Results

Ecological radiation

We reconstructed the evolution of microhabitat use across plethodontids (Fig. 1). Transitions between microhabitats occur frequently. Arboreal and bromeliad species are most frequent in the tropical clade (species 2–107 in Fig. 1). In contrast, aquatic, semi-aquatic, and saxicolous species are more common among temperate species (species 1, and 108–189 in Fig. 1).

Principal components analysis

Loadings for the morphological variables on the size-corrected PCs are shown in Table 2. With the exception of PC7 (which has zero-variance after size-correction) each PC reflects the influence of different sets of morphological variables. Larger values on PC1 indicate increasing tail length and decreasing limb length. Larger values on PC2 reflect increasing body width and decreasing limb length. PC3 reflects head length relative to other body proportions. Larger values on PC4 reflect increased snout–eye distance. PC5 reflects contrasts of snout–vent length with head length and body width. PC6 reflects the contrasting lengths of the forelimb and hindlimb. The loadings for the morphological variables on the PCs were generally similar when the PCA was performed on the tropical and temperate subgroups separately (Tables S7 and S10). Plotting PC scores for all species for the axes that explain the most shape variation shows that species do not cluster distinctly based on their microhabitat assignment and instead show extensive overlap with species utilizing different microhabitats (Fig. 2).

Phylogenetic comparative analyses

For the multivariate analysis, the BM model is preferred over the OU model, both for the analysis of all Plethodontidae and of the temperate and tropical subgroups (Table S2). For the univariate analyses, the model with estimated lambda (the strength of the phylogenetic signal; implemented in CAIC) is generally favoured, with the exception of the analysis of the entire family on PC4, temperate species on PC5, and tropical species on PC3–PC6. In the former two exceptions, the fixed-OU model is

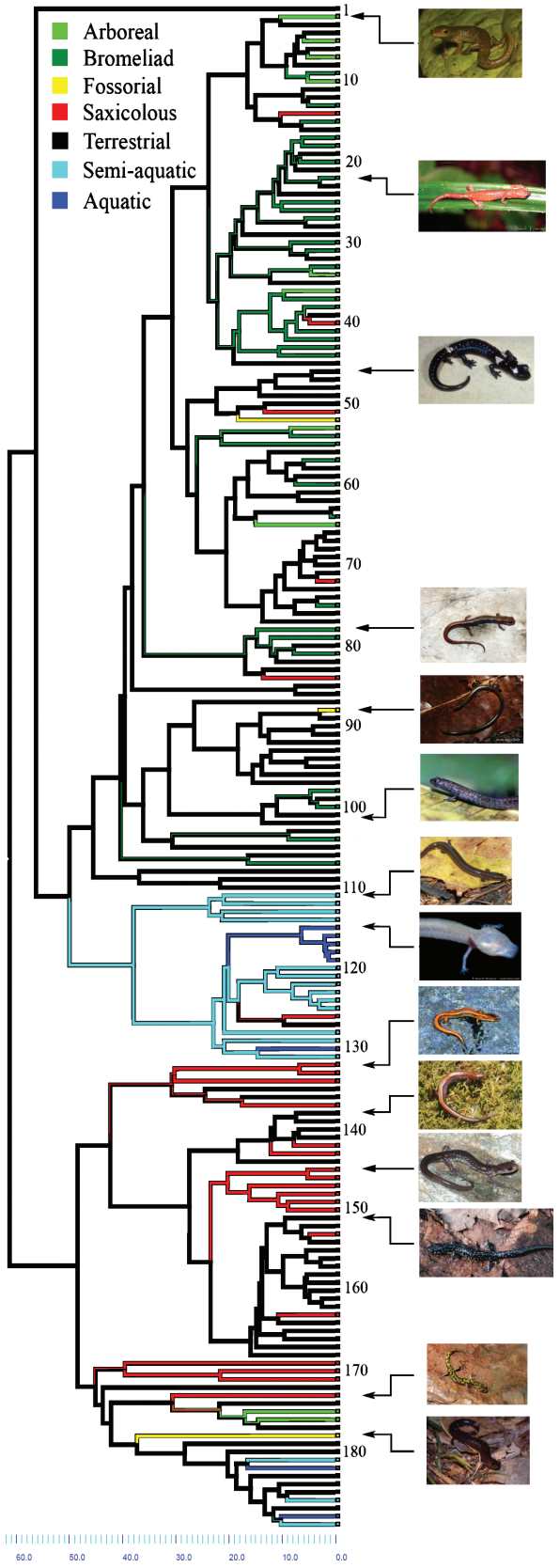


Fig. 1 Evolution of microhabitat use across the phylogeny of plethodontid salamanders. Species were assigned to one of the seven microhabitat categories following scenario 5 (with fossorial as an additional separate category). Ancestral states were estimated using maximum likelihood reconstruction under the one-rate model. Branch colours represent microhabitat use of extant species and inferred ancestors. Branches with more than one colour indicate those where there is not significant support for one state over another (difference in log likelihood units for alternate states is < 2). Arrows indicate species shown in the photographs (each representing one of the 15 clades used in Adams *et al.*, 2009). See acknowledgments for photo credits. Every tenth species is numbered. 1: *Hemidactylium scutatum*, 2: *Bolitoglossa schizodactyla*, 3: *B. colonnea*, 4: *B. adspersa*, 5: *B. medemi*, 6: *B. altamazonica*, 7: *B. peruviana*, 8: *B. palmata*, 9: *B. biseriata*, 10: *B. sima*, 11: *B. gracilis*, 12: *B. pesrubra*, 13: *B. subpalmata*, 14: *B. cerroensis*, 15: *B. minutula*, 16: *B. marmorea*, 17: *B. carri*, 18: *B. conanti*, 19: *B. diaphora*, 20: *B. dunni*, 21: *B. morio*, 22: *B. celaque*, 23: *B. synoria*, 24: *B. decora*, 25: *B. longissima*, 26: *B. porrasorum*, 27: *B. franklini*, 28: *B. lincolni*, 29: *B. dofleini*, 30: *B. engelhardti*, 31: *B. helmrichi*, 32: *Bolitoglossa rostrata*, 33: *B. hermosa*, 34: *B. riletti*, 35: *B. macrinii*, 36: *B. flaviventris*, 37: *B. platydactyla*, 38: *B. mexicana*, 39: *B. striatula*, 40: *B. yucatana*, 41: *B. lignicolor*, 42: *B. odonnelli*, 43: *B. occidentalis*, 44: *B. rufescens*, 45: *B. hartwegi*, 46: *Pseudoeurycea naucampatepetl*, 47: *P. gigantea*, 48: *P. boneti*, 49: *P. bellii*, 50: *P. cephalica*, 51: *P. galeanae*, 52: *P. scandens*, 53: *Ixalotriton niger*, 54: *I. parvus*, 55: *Parvimolge townsendi*, 56: *Lineatriton lineola*, 57: *Pseudoeurycea firscheini*, 58: *P. leprosa*, 59: *P. lynchi*, 60: *P. nigromaculata*, 61: *P. mystax*, 62: *P. werleri*, 63: *P. juarezi*, 64: *P. saltator*, 65: *P. unguidentis*, 66: *P. altamontana*, 67: *P. robertsi*, 68: *P. longicauda*, 69: *P. melanomolga*, 70: *P. gadovii*, 71: *P. cochranae*, 72: *P. anitae*, 73: *P. smithi*, 74: *P. rex*, 75: *P. exspectata*, 76: *P. brunnata*, 77: *P. goebeli*, 78: *Chiropterotriton multidentatus*, 79: *C. arboreus*, 80: *C. dimidiatus*, 81: *C. lavae*, 82: *C. priscus*, 83: *C. chondrostega*, 84: *C. magnipes*, 85: *Thorius dubitus*, 86: *T. troglodytes*, 87: *Bradytriton silus*, 88: *Oedipina pacificensis*, 89: *O. uniformis*, 90: *O. cyclocauda*, 91: *O. pseudouniformis*, 92: *O. poelzi*, 93: *O. savagei*, 94: *O. complex*, 95: *O. parvipes*, 96: *O. elongata*, 97: *O. gephyra*, 98: *Nototriton guanacaste*, 99: *N. abscondens*, 100: *N. picadoi*, 101: *N. richardi*, 102: *N. barbouri*, 103: *Dendrotriton cuchumatanus*, 104: *D. rabbi*, 105: *Nyctanolis pernix*, 106: *Cryptotriton alvarezdeltoroi*, 107: *C. nasalis*, 108: *Batrachoseps wrighti*, 109: *B. attenuatus*, 110: *B. major*, 111: *Stereochilus marginatus*, 112: *Gyrinophilus porphyriticus*, 113: *Pseudotriton montanus*, 114: *P. ruber*, 115: *Eurycea rathbuni*, 116: *E. nana*, 117: *E. sosorum*, 118: *E. neotenes*, 119: *E. tridentifera*, 120: *E. cirrigera* E, 121: *E. bislineata*, 122: *E. junaluska*, 123: *E. wilderae* J, 124: *E. cirrigera* L, 125: *E. wilderae* M, 126: *E. lucifuga*, 127: *E. longicauda*, 128: *E. quadridigitata*, 129: *E. multiplicata*, 130: *E. tynerensis*, 131: *E. spelaeus*, 132: *Plethodon vandykei*, 133: *P. idahoensis*, 134: *P. neomexicanus*, 135: *P. elongatus*, 136: *P. vehiculum*, 137: *P. dunni*, 138: *P. cinereus*, 139: *P. shenandoah*, 140: *P. nettingi*, 141: *P. hubrichti*, 142: *P. richmondi*, 143: *P. hoffmani*, 144: *P. serratus*, 145: *P. wehrlei*, 146: *P. punctatus*, 147: *P. welleri*, 148: *P. angusticlavius*, 149: *P. ventralis*, 150: *P. dorsalis*, 151: *P. glutinosus*, 152: *P. mississippi*, 153: *P. albagula*, 154: *P. grobmani*, 155: *P. montanus*, 156: *P. amplus*, 157: *P. meridianus*, 158: *P. shermani*, 159: *P. cheoah*, 160: *P. chlorobryonis*, 161: *P. teyahalee*, 162: *P. cylindraceus*, 163: *P. caddoensis*, 164: *P. ouachitae*, 165: *P. metcalfi*, 166: *P. jordani*, 167: *P. kentucki*, 168: *P. yonahlossee*, 169: *Karsenia koreana*, 170: *Hydromantes italicus*, 171: *H. platycephalus*, 172: *Ensatina eschscholtzii*, 173: *Aneides aeneus*, 174: *A. hardii*, 175: *A. lugubris*, 176: *A. ferreus*, 177: *A. flavipunctatus*, 178: *Phaeognathus hubrichti*, 179: *Desmognathus wrighti*, 180: *D. aeneus*, 181: *D. quadramaculatus* D, 182: *D. marmoratus* B, 183: *Desmognathus imitator*, 184: *D. ochrophaeus*, 185: *D. welteri*, 186: *D. fuscus* B, 187: *D. monticola* B, 188: *D. brimleyorum* and 189: *D. auriculatus*.

Table 2 Eigenvalues, percentage of variance explained, and loadings for the seven size-adjusted morphological variables on the six PCs.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	0.124	0.032	0.017	0.010	0.006	0.003
% variance explained	63	18	10	5	3	1
SVL	0.23	0.17	−0.27	**−0.31**	**−0.78**	0.02
TL	**0.81**	−0.23	0.27	0.13	0.23	0.00
HL	−0.08	−0.16	**−0.71**	−0.25	**0.48**	**0.16**
SE	−0.14	0.29	−0.21	**0.83**	−0.06	−0.11
BW	−0.12	**0.71**	**0.36**	**−0.34**	**0.31**	−0.08
FLL	**−0.36**	**−0.30**	**0.37**	0.04	−0.12	**0.70**
HLL	**−0.34**	**−0.46**	0.19	−0.12	−0.06	**−0.69**

Bold values indicate the three variables with the strongest loadings on that axis.

favoured over the two other models. For the PGLS models of the tropical species on PC3–PC6 the AIC scores of the estimated lambda model and the fixed-OU model are different by < 4. The outcomes of these two models are, however, qualitatively similar and we only show the results of the fixed-OU model. In Table S2, the AIC scores representing the fit of the PGLS models using the first microhabitat coding strategy (scenario 1) are shown. The models have similar fit under the 14 other coding strategies, hence showing these results is redundant.

In the multivariate analysis, we found a statistically significant relationship between morphology and microhabitat use for all nine categorical (nonbinary) scenarios for describing microhabitat variation (*P*-values range from < 0.0001 to 0.0361; Table 3). In contrast, we found very few significant associations between microhabitat use and the individual PCs representing shape (Tables 4 and 5). Only the relationship between microhabitat use and PC5 (snout–vent length vs. head length and body width) is significant (*P* ranges from 0.0001 to 0.0183; Table 4). This relationship is consistent with the observation that terrestrial, aquatic, and saxicolous species have a more slender body (relative to their snout–vent length) as compared to species using other microhabitats (Table S3). Excluding PC5 from the multivariate analysis (hence using PC1–PC4 and PC6 as a multivariate response variable) results in nonsignificant *P*-values for the relationship between microhabitat and shape (Table S4). Regardless of the strategy of microhabitat assignment, the PGLS models show that microhabitat explains only a small proportion of the overall morphological variation (r^2 < 0.13 for all scenarios).

When microhabitat use is treated as a binary variable (scenarios 10–15; Table 5) we find that the 'climber' (saxicolous + arboreal), bromeliad, and arboreal microhabitats are associated with PC4 (bromeliad-dwellers and arboreal and saxicolous climbers have a larger snout–eye distance) and PC5 (saxicolous species have

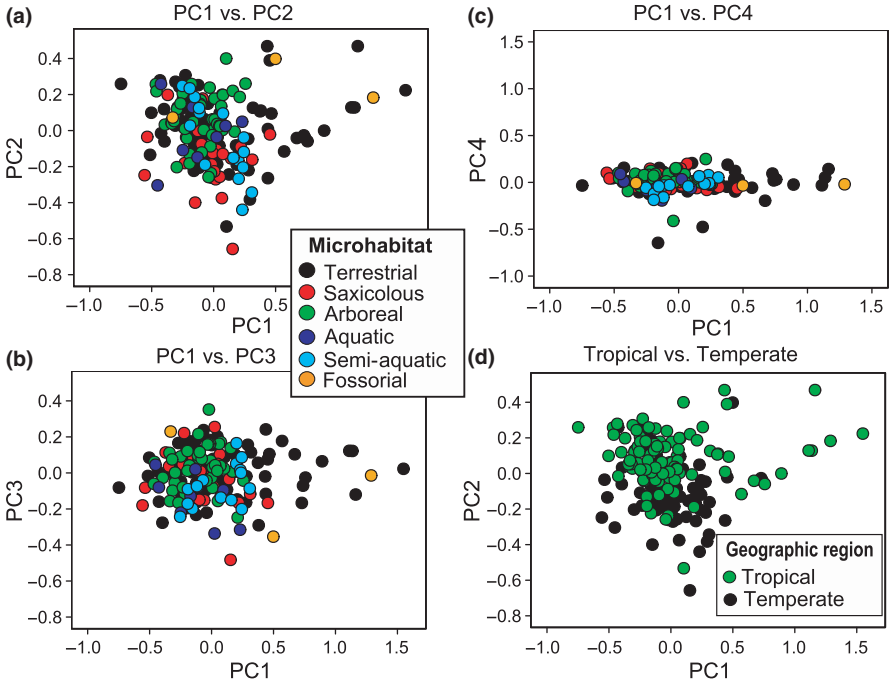


Fig. 2 Morphospace distribution from principal components analysis (PCA) on Burnaby size-corrected morphological variables for 189 plethodontid salamander species. The species scores on PC1 and PC2 (a and d), PC1 and PC3 (b), and PC1 and PC4 (c) are displayed. In (a), (b), and (c), colours are based on microhabitat assignment, but in (d), scores are coloured according to the geographic region of the species.

Table 3 Relationship between microhabitat categories and morphology, including all PCs simultaneously.

	AIC	*F*	numDf	denDf	*P*
All species (*n* = 189)					
Scenario 1	−5788.6	1.6606	30	915	**0.0148**
Scenario 2	−5786.0	1.7784	24	732	**0.0100**
Scenario 3	−5786.3	1.6448	30	915	**0.0165**
Scenario 4	−5783.9	1.5932	24	732	**0.0361**
Scenario 5	−5799.7	1.8352	36	1098	**0.0021**
Scenario 6	−5801.6	1.7461	36	1098	**0.0045**
Scenario 7	−5815.8	2.2207	36	1098	**5.63×10^{-5}**
Scenario 8	−5798.6	1.6579	30	915	**0.0151**
Scenario 9	−5781.4	1.8674	18	549	**0.0163**
Temperate species (*n* = 83)					
Scenario 1	−2655.7	0.7153	30	385	0.8670
Scenario 2	−2651.2	0.4765	24	308	0.9838
Scenario 3	−2669.2	0.7994	30	385	0.7674
Scenario 4	−2653.0	0.7913	24	308	0.7478
Scenario 6	−2656.7	0.7954	36	462	0.7978
Tropical species (*n* = 106)					
Scenario 1	−2745.6	3.3153	18	300	**9.42×10^{-6}**
Scenario 4	−2742.3	2.8052	24	400	**1.76×10^{-5}**
Scenario 6	−2746.1	3.4279	18	300	**0.0001**

PGLS was performed on PC1–PC6 after size-correction, with categorical microhabitat use as the independent variable. Different scenarios indicate different ways of describing microhabitat variation (see Table 1). Results are shown separately for the entire family, the temperate species, and the tropical species. AIC scores (AIC), F-statistics (*F*), degrees of freedom (numDf for numerator and denDf for denominator degrees of freedom) and *P*-values (*P*) are shown. Significant results after sequential Bonferroni correction are shown in boldface type.

relatively higher values for body width and head length, whereas bromeliad-dwellers and arboreal species have lower values for those variables). The only associations that are significant after Bonferroni correction are between the bromeliad microhabitat category and PC5 (scenario 13: $F_{1,187} = 21.957$; $P < 0.0001$), and microhabitat categories that involve climbing (i.e. arboreal [incl. bromeliad] and saxicolous) and PC4 (scenario 14: $F_{1,187} = 8.5602$; $P = 0.0039$; scenario 15: $F_{1,187} = 9.8756$; $P = 0.0019$). Comparing these results to the raw data (Table S1), we find that the observed association with PC5 is attributable to differences in body width relative to snout–vent length, rather than to differences in head length.

Comparable results are found when analysing the size-uncorrected variables (Table S5). After sequential Bonferroni correction, we find only PC6 (body width and head length relative to snout–vent length; corresponding to PC5 in the analysis of size-corrected data) to be significantly associated with microhabitat use (*P* ranges between 0.0001 and 0.0207, d.f. as in Table 4). As in the analysis of size-corrected variables, relatively little of the

Table 4 Relationship between microhabitat categories and morphology using separately analysed PCs, including all species.

Scenario	PC1	PC2	PC3	PC4*	PC5	PC6	Size
1							
r^2	0.011	0.032	0.015		0.106	0.052	0.005
λ	0.964	0.868	0.647		0.623	0.674	0.899
$F_{4,184}$	0.528	1.504	0.703	2.2589	5.435	2.515	0.243
P	0.716	0.203	0.591	0.0645	**0.0004**	0.043	0.914
2							
r^2	0.011	0.012	0.010		0.106	0.014	0.005
λ	0.964	0.873	0.659		0.624	0.717	0.905
$F_{3,185}$	0.692	0.773	0.613	2.7442	7.279	0.895	0.305
P	0.558	0.511	0.607	0.0445	**0.0001**	0.445	0.822
3							
r^2	0.005	0.047	0.012		0.104	0.023	0.007
λ	0.962	0.846	0.655		0.618	0.710	0.908
$F_{4,184}$	0.254	2.251	0.554	2.3223	5.356	1.088	0.340
P	0.907	0.065	0.697	0.0584	**0.0004**	0.364	0.851
4							
r^2	0.011	0.033	0.015		0.040	0.052	0.001
λ	0.962	0.865	0.649		0.648	0.672	0.906
$F_{3,185}$	0.700	2.115	0.955	2.7338	0.055	3.370	0.042
P	0.553	0.100	0.415	0.0451	0.055	0.020	0.989
5							
r^2	0.012	0.034	0.050		0.128	0.067	0.012
λ	0.965	0.870	0.659		0.635	0.689	0.896
$F_{5,183}$	0.452	1.274	1.946	1.7898	5.350	2.609	0.437
P	0.811	0.277	0.089	0.1170	**0.0001**	0.026	0.822
6							
r^2	0.011	0.034	0.021		0.071	0.058	0.020
λ	0.962	0.866	0.653		0.594	0.691	0.909
$F_{5,183}$	0.399	1.302	0.772	1.4696	2.802	2.237	0.763
P	0.849	0.265	0.571	0.2018	**0.018**	0.053	0.577
7							
r^2	0.005	0.020	0.060		0.124	0.039	0.015
λ	0.968	0.877	0.717		0.648	0.715	0.901
$F_{5,183}$	0.184	0.752	2.347	1.9084	5.187	1.488	0.549
P	0.968	0.585	0.043	0.1108	**0.0002**	0.196	0.739
8							
r^2	0.012	0.033	0.025		0.084	0.056	0.005
λ	0.966	0.871	0.664		0.589	0.667	0.905
$F_{4,184}$	0.575	1.547	1.176	3.0786	4.242	2.719	0.225
P	0.681	0.191	0.323	0.0484	**0.003**	0.031	0.924
9							
r^2	0.001	0.011	0.010		0.088	0.011	0.001
λ	0.963	0.863	0.648		0.631	0.726	0.907
$F_{2,186}$	0.121	1.064	0.916	1.7506	8.973	1.055	0.123
P	0.886	0.347	0.402	0.1252	**0.0002**	0.350	0.884

PGLS was performed on all seven PCs after size-correction, with categorical microhabitat use as the independent variable. Different scenarios indicate different ways of describing microhabitat variation (see Table 1). Percentage of variance explained (r^2), phylogenetic signal (λ), *F*-statistics (*F* with degrees of freedom in subscript), and *P*-values (*P*) are shown. Significant results after sequential Bonferroni correction are shown in boldface type.

* Analysis performed using (fixed-OU) model from R-package 'nlme' that returns no values for r^2 and λ.

Table 5 Relationship between binary microhabitat categories and morphology among all 189 sampled plethodontid species.

Scenario	PC1	PC2	PC3	PC4*	PC5	PC6	Size
10 (terrestrial)							
r^2	0.004	0.001	0.000		0.018	0.000	4.411×10^{-5}
λ	0.965	0.881	0.675		0.636	0.731	0.906
r	0.024	−0.010	0.002	−0.0175	−0.019	0.002	−0.009
$F_{1,187}$	0.738	0.241	0.011	1.5156	3.451	0.059	0.008
P	0.392	0.624	0.916	0.2198	0.065	0.809	0.928
11 (aquatic)							
r^2	0.008	0.012	0.009		0.007	0.013	0.000
λ	0.962	0.875	0.656		0.611	0.721	0.907
r	−0.139	0.102	−0.070	−0.0157	−0.036	0.030	0.096
$F_{1,187}$	1.570	2.275	1.729	0.1483	1.321	2.375	0.073
P	0.212	0.133	0.190	0.7006	0.252	0.125	0.788
12 (saxicolous)							
r^2	0.002	0.000	0.000		0.022	0.001	0.002
λ	0.965	0.879	0.679		0.579	0.725	0.904
r	−0.027	−0.008	0.006	0.0074	−0.033	0.005	0.099
$F_{1,187}$	0.321	0.063	0.047	0.1301	4.298	0.223	0.373
P	0.571	0.803	0.829	0.7187	0.040	0.637	0.542
13 (bromeliad)							
r^2	0.001	0.001	0.009		0.105	0.004	0.008
λ	0.964	0.880	0.677		0.648	0.727	0.899
r	−0.018	−0.011	−0.029	0.0399	0.061	0.007	−0.158
$F_{1,187}$	0.273	0.187	1.658	4.6126	21.957	0.794	1.581
P	0.602	0.666	0.199	0.0330	**5.36×10^{-6}**	0.374	0.210
14 (climber)							
r^2	0.004	0.001	0.004		0.002	0.000	0.001
λ	0.963	0.879	0.687		0.611	0.727	0.906
r	−0.028	−0.007	0.016	0.0446	0.007	0.001	0.052
$F_{1,187}$	0.793	0.114	0.687	8.5602	0.411	0.045	0.228
P	0.374	0.736	0.408	**0.0039**	0.522	0.833	0.634
15 (arboreal)							
r^2	0.002	0.000	0.003		0.021	0.001	1.09×10^{-5}
λ	0.963	0.879	0.684		0.605	0.733	0.906
r	−0.022	0.007	0.016	0.0584	0.028	−0.003	−0.006
$F_{1,187}$	0.351	0.070	0.500	9.8756	4.094	0.164	0.002
P	0.555	0.791	0.480	**0.0019**	0.044	0.686	0.964

PGLS was performed on all seven PCs after size-correction, with binary categorized microhabitat use as the independent variable. Different scenarios indicate different ways of describing microhabitat variation (see Table 1). Percentage of variance explained (r^2), phylogenetic signal (λ), regression coefficients (r), F-statistics (F, degrees of freedom in subscript), and P-values (P) are shown. Significant results after sequential Bonferroni correction are shown in boldface type.

* Analysis performed using (fixed-OU) model from R-package 'nlme' that returns no values for r^2 and λ.

overall variation in morphology is explained by microhabitat ($r^2 < 0.12$ for all scenarios).

Using phylogenetic trees with younger and older root ages (i.e. 48 and 69 Myr) did not influence the overall results of our analyses. Similar to our findings described above, we find only PC5 to be significantly associated with microhabitat use (Tables S6 and S7).

Tropical and temperate subgroups

For the temperate species, very few significant relationships between microhabitat use and body shape are evident and no statistically significant association between microhabitat use and size was found (Tables 3, S9 and S10). The multivariate analysis revealed no significant associations between microhabitat and morphology in temperate species (Table 3). Similarly, in all but one scenario, no significant associations are found between microhabitat use and the individual shape PCs. The single exception involves the association between microhabitat and PC2 for scenario 3 ($F_{4,78} = 6.81$; $P < 0.0001$; Table S9). However, for this scenario, in which fossorial microhabitat use is considered separately (instead of combined with, for example, terrestrial microhabitat use), only one species was categorized as fossorial in the temperate subgroup (Table S1).

For the tropical subgroup, results are very similar to those across the family. The multivariate analysis shows a strong relationship between microhabitat use and shape for all scenarios (P-values from < 0.0001 to 0.0010; Table 3), but not for the microhabitat-size association. Exploring the PCs individually reveals a significant association between PC3 (head length and snout–eye distance vs. body width) and microhabitat use (categorical scenarios 1 and 4 and binary scenario 'bromeliad' [13]; P-values range between 0.0014 and 0.0233). Also, microhabitat use (all categorical scenarios and binary scenarios 'terrestrial' [10] and 'bromeliad' [13]) is significantly correlated with PC5 (body width relative to snout–vent length; P-values range between 0.0061 and 0.0176; Table S12). Therefore, the relationship between morphology and microhabitat seems to be related to differences in snout–vent length and body width between arboreal (broader) and terrestrial (slender) species. In summary, our results show that there is little relationship between morphology and microhabitat in plethodontid salamanders, and the relationship that is present is driven primarily by shape variation in the tropical clade alone.

Discussion

A major paradigm in evolutionary biology and ecology is that the morphology of species often reflects adaptation to the microhabitats in which they occur (e.g. Wainwright & Reilly, 1994). This relationship has been shown in numerous studies, particularly those focusing on adaptive radiation (e.g. Schluter, 2000). Our results from plethodontid salamanders offer an interesting counter-example. Although we do find a significant relationship between variation in morphology and microhabitat when including all 189 species, this relationship seems to be largely attributable to variation in a few morphological traits and only within the relatively recent but species-rich tropical clade (supergenus *Bolitoglossa*). In

contrast, among the temperate species (which include all four subfamilies and nearly all microhabitats), the relationship between morphology and microhabitat is largely absent, both when shape is included as a multivariate dependent variable and as separate univariate variables. Additionally, across the entire family, most individual PCs representing different aspects of shape show no relationship with microhabitat use (and those that do explain very little of the overall morphological variation, with $r^2 < 0.13$). Also, size and microhabitat are unrelated across all plethodontids and in separate analyses of the temperate and tropical subgroups. In short, variation in the tropical clade seems to explain most of the observed relationships between morphology and microhabitat categories in plethodontids, despite a remarkable ecological radiation among the temperate species (Fig. 1), encompassing nearly all possible microhabitats for nonmarine vertebrates (e.g. aquatic, arboreal, fossorial, saxicolous, terrestrial).

In theory, the limited relationships observed between morphology and microhabitat could be an artifact of any one of several factors, but most of these seem unlikely. One of these factors is measurement error. However, the morphometric data of Adams *et al.* (2009) show strong repeatability between measurers ($r = 0.99$), suggesting that the data are generally accurate. Furthermore, the morphological shape variables generally show a strong phylogenetic signal (typically $\lambda \sim 0.9$ in the univariate analyses [Table 5]). This result indicates that the morphological variation tracks the phylogeny (and is therefore nonrandomly distributed among species), but nevertheless fails to track changes in microhabitat. Another possibility is that our study had insufficient statistical power. However, we utilized a large sample size of species ($n = 189$ for the entire family and $n = 83$ and 106 for the temperate and tropical subgroups, respectively). Additionally, even though only ~45% of plethodontid species were included, our sampling includes nearly all genera (with the exception of the recently described *Urspelerpes*; AmphibiaWeb, 2011). Thus, it seems likely that most of the major variation in microhabitat and morphology within plethodontids was included. We acknowledge that there could (in theory) be stronger ecomorphological relationships in salamanders outside plethodontids. However, except for a few elongate, paedomorphic genera in other families (e.g. *Amphiuma*, *Proteus*, *Pseudobranchus*, *Siren*), salamander body forms in other families are generally similar to those in plethodontids (e.g. Wiens & Hoverman, 2008). There are also many potential ambiguities in assigning species to microhabitats, but we employed a large number of alternative coding schemes, none of which show strong relationships between morphology and microhabitat. However, we acknowledge that there could be other aspects of microhabitat that are important but not reflected in our coding. Nevertheless, it seems that morphology should generally track these coarse microhabitat categories rather than finer distinctions within them (e.g. we postulate that the important distinction for morphology should be between microhabitats that require salamanders to climb vs. burrow vs. swim, and that minor differences in, for example, arboreal microhabitats should be less relevant for explaining morphological variation). Also, although it would be interesting to analyse the effect of within-category variation on morphology, this may require additional data on microhabitat utilization beyond what are currently available for most species.

Perhaps the most important limitation of our study is that it can only reflect the morphological variables that we measured. Nevertheless, studies of lizards (which are similar in general body form) suggest that microhabitat can strongly influence the relative proportions of the head, limbs, trunk, and tail (e.g. Losos, 1990a,b; Vitt *et al.*, 1997; Irschick & Losos, 1998; Glor *et al.*, 2003; Wiens *et al.*, 2006). Thus, the variables that we used are expected to be particularly important for locomotion in different microhabitats. Nevertheless, there may still be important variation in other morphological variables we did not take into account in the present study that may be related to microhabitat (e.g. foot shape and interdigital webbing; Jaekel & Wake, 2007; Adams & Nistri, 2010; or head shape: Adams, 2004, 2010). Similarly, aspects of shape morphology that are not captured in one of the six PCs but are in fact the result of ecomorphological adaptations might not be identified as such. This is a major caveat in the use of individual PCs to describe morphological variation, as compared to using approaches such as factor analysis (for a discussion of factor analysis in morphometrics see Blackith & Reyment, 1971; Mitteroecker & Bookstein, 2007). However, since these PCs were identified by PCA as the major gradients of variation, it seems unlikely that morphological adaptations to microhabitats have occurred in other directions in morphospace that are not represented by these PCs. Finally, even if there is important ecomorphological variation in other traits (or in other ways of looking at these same traits), the generally weak relationship between microhabitat and the major body proportions that we included is still remarkable.

The lack of relationship between morphological variation and microhabitat use in temperate plethodontids is most surprising when contrasted with lizards (nonsnake squamate reptiles). Most lizards have body forms that are similar to those in salamanders and many studies show strong relationships between morphology and habitat (e.g. Losos, 1990a,b; Vitt *et al.*, 1997; Irschick & Losos, 1998; Glor *et al.*, 2003; Wiens *et al.*, 2006; but see Vanhooydonck & Van Damme, 1999; Schulte *et al.*, 2004). Why are similar relationships weak or absent in plethodontids? Below, we suggest a few tentative hypotheses, but we acknowledge that these are highly speculative and will require additional work to evaluate.

One hypothesis is that the strong relationships between morphology and microhabitat use in lizards are not replicated in salamanders because of major differences in behaviour and/or physiology between lizards and salamanders. For example, both laboratory and field studies in lizards show that longer limbs increase maximal sprinting speed and acceleration, and facilitate predator escape behaviour (Irschick & Jayne, 1998; Irschick & Losos, 1998; Irschick, 2002). However, it is not clear that plethodontids generally sprint away from predators as lizards do. For example, many *Plethodon* exhibit an immobile posture when disturbed, presumably to be less conspicuous to visually oriented predators (e.g. Dodd, 1990). Further, plethodontids are typically slow moving and have low metabolic rates relative to other amphibians and reptiles (Vitt & Caldwel, 2009). Thus, plethodontids may generally be more homogeneous in their locomotor performance in different environments. Alternately, plethodontids may differ significantly in their performance in different environments due to differences in behaviour and physiology, but without corresponding differences in morphology. These two hypotheses could be tested by comparing the performance of plethodontid species that occur in different environments at different ecologically relevant activities (e.g. climbing, terrestrial movement, swimming). If the first hypothesis is correct, species with similar morphology should have similar performance at these different activities, regardless of the microhabitat in which they typically occur. If the second hypothesis holds, there should be strong relationships between the microhabitat of species and their performance at these activities (e.g. arboreal species should be superior climbers, aquatic species should be superior swimmers), even if there are few morphological differences among species.

A related hypothesis that might explain the weak relationship between morphology and microhabitat is that there are developmental constraints on morphological evolution in plethodontids which limit their adaptation to different microhabitats. In fact, plethodontids are well known for their general morphological conservatism (e.g. rates of morphological evolution are decoupled from rates of species diversification; Adams *et al.*, 2009). We can think of two potential examples of how developmental constraints might limit ecomorphological evolution in plethodontids, although both examples are also somewhat problematic. First, vertebral number is fixed in most tropical bolitoglossines (Wake, 1966), which may constrain their evolution of body shape (but see Parra-Olea & Wake, 2001). However, we note that tropical bolitoglossines also show the strongest relationships between morphology and microhabitat in our study. Second, species with an aquatic larval stage and a terrestrial adult stage must be able to move both in water and on land. Given that the basic body form is established prior to metamorphosis in plethodontids (e.g. head, trunk, limb, and tail proportions), then the need for the same body shape to function on both land and in water may constrain adaptation of adult morphology to particular microhabitats. However, it should be noted that most plethodontines (excluding most *Desmognathus*) and all bolitoglossines have direct development and therefore lack an aquatic larval stage (Vitt & Caldwel, 2009). In summary, plethodontids show surprisingly little relationship between morphology and microhabitat categories, but determining the causes of this pattern will require more detailed studies of their behaviour, performance, morphology, and development.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 R codes.

Appendix S2 Supplementary material:

Table S1 Microhabitat assignment, geographic region, and species means for the seven morphological variables for 189 plethodontid salamander species.

Table S2 Comparison of AIC scores for the different models of character evolution.

Table S3 Correlation coefficients of microhabitat categories for Microhabitat∼PC5.

Table S4 Multivariate PGLS on relationship between microhabitat use and morphology, using PC1–PC4 and PC6 as response variable (i.e. excluding PC5).

Table S5 Relationship between microhabitat and size-uncorrected morphological variables.

Table S6 Relationship between microhabitat and morphology using the phylogeny with a root age of 48 Myr.

Table S7 Relationship between microhabitat and morphology using the phylogeny with a root age of 69 Myr.

Table S8 Eigenvalues, percentage variance explained, and loadings for the morphological variables for PC1–PC6 for the temperate plethodontid species.

Table S9 Relationship between microhabitat use (scenarios 1–6) and morphology for temperate species.

Table S10 Relationship between microhabitat use (scenarios 10–15) and morphology for temperate species.

Table S11 Eigenvalues, percentage variance explained, and loadings for the morphological variables for the tropical subgroup on PC1–PC6.

Table S12 Relationship between microhabitat use and morphology for tropical species.

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