Digit reduction, body size, and paedomorphosis in salamanders

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SUMMARY   The loss of digits is a widespread evolutionary trend in tetrapods which occurs in nearly every major clade. Alberch and Gale showed that the order in which digits are evolutionarily lost in salamanders versus frogs corresponds to the order in which they develop in each group, providing a classic example of developmental constraint. However, what actually drives the loss of digits in salamanders has remained unclear. Alberch and Gale suggested that loss of digits might be associated with paedomorphosis or with reduced body size. We test these hypotheses by combining morphometric and phylogenetic information for 98 species of salamanders. We find that digit loss is associated with both paedomorphosis and reduction in body size. However, these trends are surprisingly contradictory, in that paedomorphosis is significantly associated with an increase in body size in salamanders. Thus, much of the extreme digit reduction is found in the smaller species within paedomorphic clades that have, on average, unusually large body size. Our results show that the consequences of changes in body size on morphology are highly context dependent. We also show (possibly for the first time) a significant association between paedomorphosis and increased body size, rather than the expected association with reduced body size.

INTRODUCTION

Digit reduction, defined here as the evolutionary loss of one or more fingers or toes, is a pervasive trend in tetrapod evolution, occurring in nearly every major clade (e.g., frogs, salamanders, turtles, squamates, dinosaurs, mammals, crocodilians; Alberch and Gale 1985; Carroll 1988). The gain and loss of digits in tetrapods offers an important model system for evolutionary studies (e.g., Lande 1978; Wake 1991; Shubin et al. 1997; Galis et al. 2001; Wiens and Slingluff 2001; Shapiro et al. 2003; Crumly and Sánchez-Villagra 2004; Stopper and Wagner 2005; Kohlsdorf and Wagner 2006), and many textbook examples of evolutionary processes and phenomena come from this system (e.g., Futuyma 2005, p. 491). For example, digit reduction in amphibians was the subject of a classic study of developmental constraints in evolution. Alberch and Gale (1985) showed that digits develop in a different sequence in frogs and salamanders and that these differences in developmental sequence seem to correspond to the evolutionary order in which digits are lost on the hindlimb in each group, with the last digit to develop being the digit most likely to be lost. These authors also showed that experimental perturbations of development in selected frogs and salamanders (i.e., reducing limb-bud size with mitotic inhibitors) generated the morphologies that were expected for each group based on the observed patterns of digit loss among species (see also Stopper and Wagner 2006).

The study by Alberch and Gale (1985) helped illuminate why digits are lost in different sequences in different clades of amphibians. But why should digits be lost in the first place? In other words, why have some lineages lost one or more digits whereas others retain a full complement? Alberch and Gale (1985) suggested two hypotheses, stating that digit reduction was associated with either "global developmental truncation associated with dwarfism or a slowdown in the rate of cell proliferation associated with paedomorphosis" (p. 18). In this paper, we test these hypotheses in salamanders, utilizing new phylogenies, new morphometric data, and phylogenetic comparative methods. Specifically, we test if digit loss is associated with reductions in overall body size or with paedomorphosis.

Intuitively, these two factors could also be related to each other, given that there are many species with small body size that exhibit other paedomorphic traits (e.g., Alberch and Alberch 1981; Yeh 2002). However, in salamanders there are also species that are traditionally considered to be paedomorphic (because they fail to undergo metamorphosis from aquatic larvae to a terrestrial adult stage) but which have relatively large body sizes, including the largest extant salamanders (*Andrias* and *Cryptobranchus*; Pough et al. 2004). Surprisingly, the relationship between body size and paedomorphosis in salamanders has never been tested using statistical phylogenetic methods.

Evolution of body shape and relative limb size may also be important for explaining patterns of digit reduction among

species. For example, in squamate reptiles, digit reduction is widespread among families, and seems to be associated with a reduction in the relative size of the limb (e.g., Lande 1978; Greer 1991; Greer et al. 1998; Wiens and Slingluff 2001; Wiens et al. 2006). Reduction in relative limb size is in turn associated with body elongation (i.e., an increase in body length relative to body width or head length), leading to an overall snake-like body form (e.g., Lande 1978; Greer et al. 1998; Wiens and Slingluff 2001). Although some digit-reduced salamanders also appear to have an elongate, snake-like body form (e.g., *Amphiuma, Siren*; Pough et al. 2004), the relationships between digit reduction, limb reduction, and body elongation have never been quantitatively tested in salamanders. We test these relationships here.

Our study focuses specifically on salamanders, but the processes that drive digit evolution may be important in many groups of tetrapods. Furthermore, the impacts of body size and paedomorphosis on patterns of morphological evolution are potentially relevant to all multicellular organisms, but these factors are only rarely explored using statistical phylogenetic methods (e.g., Yeh 2002).

METHODS

Morphometric data

We obtained morphometric data from 203 species of salamanders (roughly 40% of all described species; AmphibiaWeb 2007), including representatives of all 10 families and most genera. Throughout the paper we follow the standard taxonomy of Amphibiaweb (2007; checked November 2007). Measurements were taken from alcohol-preserved museum specimens, primarily from the Carnegie Museum of Natural History (CM). Specimens examined are listed in Appendix A, and the raw data are summarized in Appendix B. We attempted to include only adult specimens of each species. However, sexual maturity can be difficult to determine in salamanders (without dissection) and so we included only those individuals that appeared to be adults based on having body lengths similar to the largest individuals examined within a species, and based on published descriptions of adult body sizes (e.g., Petranka 1998). Thus, in many cases, the sample sizes for each species listed in Appendix B are an underestimate of the actual number of specimens examined because only suitable specimens are listed. We did not distinguish between males and females, as sexing individuals was often difficult and sexual dimorphism in size and shape in salamanders seems to be limited (e.g., Duellman and Trueb 1986, p. 53). For example, Petranka (1998) provided data on body size for all North American salamanders (including representatives of all families but Hynobiidae). He mentioned female-biased sexual-size dimorphism of 0–15% in most species (i.e., female body size exceeds male body size by 15% of male body size), but most importantly, he reported that the range in adult body size from largest to smallest individuals is close to or above 100% (twofold) in most species. This pattern suggests that sexual dimorphism is a minor source of variation in adult body sizes among individuals within species. All measurements were made by a single individual (J. T.

H.) within a period of approximately 12 months, primarily using digital calipers (taken to the nearest 0.01 mm). For some very large specimens, a yardstick was used.

Nine measurements were taken from all specimens: (1) SVL; snout–vent length; tip of snout to posterior end of cloaca; (2) TL; tail length; posterior end of cloaca to tail tip; (3) HL; head length; tip of snout to posterior corner of jaw (externally visible); (4) SE; snout-eye length; tip of snout to posterior corner of eye; (5) BW; body width; measured just posterior to forelimb insertion; (6) FLL; forelimb length; posterior insertion of forelimb to outstretched tip of longest (usually the third) finger; (7) HLL; hindlimb length; posterior insertion of hindlimb to outstretched tip of longest (usually the fourth) toe; (8) number of fingers; and (9) number of toes. Some rare individuals showed asymmetric variation in digit number; these were not included in summaries of digit numbers for species. Following Alberch and Gale (1985), we focus only on the loss of entire digits (digit reduction), rather than losses of individual phalanges from digits (phalangeal reduction). Note that although our data on digit numbers are entirely external, we are familiar with the osteology of most of the included species (i.e., Wiens et al. 2005), and have confirmed that the digit numbers determined externally correspond to digit numbers based on cleared-and-stained skeletal preparations.

Phylogeny

Morphological data were analyzed in a phylogenetic context. The phylogeny used was a chronogram (a tree with branch lengths proportional to the estimated ages of lineages) that was combined from two recent literature sources. The first (from Wiens 2007) was based on a Bayesian analysis of 66 salamander taxa (representing all families) based on DNA sequences of the slow-evolving nuclear gene, recombinase activating protein (RAG-1), taken from several literature sources (see Wiens [2007] for original sources). The chronogram was estimated using penalized likelihood (Sanderson 2002), based on 11 fossil calibration points (Wiens 2007). This phylogeny is similar to a widely used higher-level salamander phylogeny (Larson and Dimmick 1993) based on morphology and ribosomal RNA sequences, and to a recent analysis based on combined RAG-1 sequences, ribosomal RNA sequences, and non-molecular data (Wiens et al. 2005). This higher-level phylogeny lacked detailed taxon sampling within bolitoglossine plethodontids, a clade containing nearly half of all salamander species (Amphibiaweb 2007). To address this gap, we used a second phylogeny that included 158 species of bolitoglossine salamanders (and outgroups), based mostly on previously published DNA sequences of the mitochondrial 16S and cytochrome *b* genes (Wiens et al. 2007). This phylogeny was converted to a chronogram using penalized likelihood analysis (see Wiens et al. 2007 for details), using the age of bolitoglossines inferred from the previous analysis as a root age. The phylogenies and branch lengths from the two analyses were then manually combined into a single tree file. The end result was a phylogeny for 210 salamander taxa, all with comparable branch lengths using the common currency of clade age (following Wiens et al. 2006). Overall, most branches of these trees were relatively strongly supported based on parsimony bootstrap values and Bayesian posterior probabilities in these previous studies (Wiens 2007; Wiens et al. 2007).

We obtained morphometric data for 203 species and phylogenetic information for 210 taxa, but these two sets of taxa were not fully overlapping. There were many species for which we had morphometric data but no phylogenetic data and many for which we had phylogenetic data but no morphometric data. Strictly overlapping data were available for 90 taxa. For several additional species, we used morphometric data for a congeneric species when we had phylogenetic data for one member of a genus but morphometric data for a different species (in parentheses): Plethodontidae: *Dendrotriton cuchumatanus* (*D. xolocalcae*), *Hydromantes brunus* (*H. platycephalus*); Salamandridae: *Pachytriton labiatus* (*P. brevipes*); Sirenidae: *Pseudobranchus axanthus* (*Pseudobranchus striatus*). For the plethodontid genus *Thorius*, three species were exchanged based on the presumed phylogeny within the genus (Hanken 1983); *T. dubitus* (*T. pennatulus*), *T. minutissimus* (*Thorius macdougalli*), *Thorius sp.* (*Thorius pulmonaris*). We also used morphometric data for the salamandrid *Mertensiella caucasica* for the species *Lyciasalamandra luschani* in the phylogeny; although these are not sister taxa in the context of all salamandrids (Weisrock et al. 2006b), they are equivalent in terms of the salamandrid species included in our phylogeny (i.e., both are more closely related to *Salamandra* than to any other included genus, but note that our placement of *Chioglossa* based on nuclear data differs from the mitochondrial tree of Weisrock et al. 2006b). In general, replacing species in this manner should have little or no impact on the branch lengths (e.g., given that only one *Pseudobranchus* is included and branch lengths reflect time, the length of this branch will be the same regardless of which species is used). In total, we analyzed morphometric data from 98 species in a phylogenetic context (Fig. 1).

Although we did not include all species or even genera of salamanders in these analyses of 98 species, our direct examination of >200 species (and our knowledge of the literature) strongly suggests that we have captured most of the major transitions in digit number and body form within salamanders. Three genera in which one toe is absent were not included because appropriate branch length information was lacking (the hynobiids *Batrachuperus* and *Paradactylodon* and the salamandrid *Salamandrina*), but similar losses occur in many other genera that were included (i.e., four-toed taxa that are not unusually miniaturized or paedomorphic, such as *Eurycea quadridigitata, Hemidactylium scutatum*, and *Salamandrella keyserlingii*).

In theory, additional taxa could have been included, given that phylogenetic information is available for many more species. However, branch lengths are critically important for our comparative analyses, and so we focused primarily on these 98 taxa because both morphometric data and comparable branch lengths were available for them.

Comparative analyses

To account for the phylogenetic relatedness of species, most analyses of the relationships between variables were conducted using independent contrasts (Felsenstein 1985). The raw data for these analyses were average values across individuals within a species. Variables were natural-log transformed before analysis, and to accommodate some traits that had values of zero in some taxa (e.g., hindlimb length in sirenids), the value of one was added to each variable before log transformation.

We tested for relationships between digit number and (1) overall body size, (2) absolute limb length, and (3) relative limb length. We also tested for a relationship between relative limb length and body elongation. Studies of body-form evolution in squamates have often divided limb-length and body-length measurements by head length to achieve indices of relative limb length and body elongation (e.g., Lande 1978; Caputo et al. 1995), given the assumption that the size and shape of the head shows relatively little variation relative to the dramatic differences in limb and body length. Similarly, we used residuals from regression of independent contrasts of head length against contrasts of limb length to create indices of relative limb length, and head length versus total length (SVL+TL) for indices of elongation (following Wiens and Slingluff 2001). A similar approach would be to use body width in place of head length. However, there is a strong correlation between head length and body width for the 98 sampled species ($r = 0.981$), suggesting that use of body width in place of head length would yield similar results.

Analyses using residuals may be affected by the frequency of different morphologies among the sampled species. Therefore, we also conducted a parallel set of analyses using ratios of head length to forelimb, hindlimb, and total length as indices of limb length and body elongation. Although ratios also present problems for statistical analysis, they will be unaffected by the frequency of different morphologies among species.

To obtain a multivariate index of overall body size, we performed principal components analysis (PCA) in Statview, using all variables except digit numbers. The first principal component (PC1) appeared to reflect body size, in that all variables loaded positively and more-or-less evenly. Therefore, we used the scores of individual species on PC1 as our primary index of body size. We also used total length and body width (both ln-transformed) as measures of body size, but these indices gave similar results to those using PC1 (J. J. Wiens, unpublished).

Independent contrasts were calculated using COMPARE version 4.6b (Martins 2004). Branch lengths were taken from the chronogram. For all regression analyses using independent contrasts, the intercept was forced through zero. Statistical analyses were implemented using Statview, version 4.1 (Abacus Concepts, Berkeley, CA, USA).

We acknowledge that the most relevant variable relating limb or body size to digit number would be the absolute size of the limb bud during that period of development during which digit number is determined (at least based on the hypothesis of Alberch and Gale 1985). However, these embryonic morphometric data would be very difficult to obtain for many taxa. Although our data from adults cannot address whether limb buds were small or large in taxa with large adult limb sizes, it seems safe to assume that taxa having small limbs as adults are unlikely to have large limb buds (in terms of absolute size) during development.

We tested for a relationship between digit reduction and paedomorphosis (as defined below) using phylogenetic generalized least squares (PGLS; Martins and Hansen 1997), as implemented in COMPARE 4.6b. We tested for a relationship between the continuous dependent variables, finger and toe numbers (average for each digit within each species), and the binary independent variable, paedomorphosis. We assessed significance using standard tables for r (Rohlf and Sokal 1995), following Ord and Martins

					Taxon
Hynobiidae		4	5	T	98.7	*Hynobius nebulosus*
		4	4	T	77.8	*Salamandrella keyserlingii*
		4	5	T	140.5	*Onychodactylus japonicus*
Cryptobranchidae		4	5	P	476.8	*Cryptobranchus alleganiensis*
Sirenidae		4	5	P	501.5	*Andrias davidianus*
		3	0	P	149.6	*Pseudobranchus axanthus*
		4	0	P	297.3	*Siren intermedia*
Dicamptodontidae		4	5	T	154.8	*Dicamptodon tenebrosus*
		4	5	P	137.0	*Dicamptodon copei*
Ambystomatidae		4	5	T	163.6	*Ambystoma gracile*
		4	5	T	108.4	*Ambystoma opacum*
		4	5	P	198.2	*Ambystoma ordinarium*
Salamandridae		4	5	T	119.2	*Chioglossa lusitanica*
		4	5	T	187.4	*Salamandra salamandra*
		4	5	T	151.1	*Mertensiella caucasica*
		4	5	T	153.3	*Taricha rivularis*
		4	5	T	116.2	*Notophthalmus viridescens*
		4	5	T	189.2	*Pachytriton labiatus*
		4	5	T	97.9	*Triturus pygmaeus*
		4	5	T	109.3	*Calotriton asper*
Proteidae		3	2	P	196.5	*Proteus anguinus*
		4	4	P	190.5	*Necturus alabamensis*
		4	4	P	267.0	*Necturus maculosus*
Rhyacotritonidae		4	5	T	71.8	*Rhyacotriton variegatus*
		4	5	T	78.2	*Rhyacotriton cascadae*
		4	5	T	72.0	*Rhyacotriton kezeri*
Amphiumidae		3	3	P	702.3	*Amphiuma tridactylum*
		2	2	P	654.2	*Amphiuma means*
		1	1	P	212.6	*Amphiuma pholeter*
		4	4	T	88.0	*Hemidactylium scutatum*
		4	5	T	107.3	*Stereochilus marginatus*
		4	5	T	132.5	*Pseudotriton ruber*
		4	5	T	164.8	*Gyrinophilus porphyriticus*
		4	5	T	79.8	*Eurycea multiplicata*
		4	5	T	101.6	*Eurycea bislineata*
		4	5	T	151.3	*Eurycea longicauda*
		4	4	T	70.7	*Eurycea quadridigitata*
		4	5	P	65.3	*Eurycea neotenes*
		4	5	T	97.6	*Plethodon vandykei*
		4	5	T	105.6	*Plethodon elongatus*
		4	5	T	101.9	*Plethodon vehiculum*
		4	5	T	88.0	*Plethodon cinereus*
		4	5	T	69.8	*Plethodon serratus*
		4	5	T	75.1	*Plethodon welleri*
		4	5	T	112.4	*Plethodon ouachitae*
		4	5	T	157.5	*Plethodon yonahlossee*
		4	5	T	110.0	*Ensatina eschscholtzii*
		4	5	T	85.1	*Hydromantes platycephalus*
		4	5	T	140.7	*Aneides lugubris*
		4	5	T	108.8	*Aneides aeneus*
		4	5	T	196.7	*Phaeognathus hubrichti*
		4	5	T	47.5	*Desmognathus wrighti*
		4	5	T	116.4	*Desmognathus monticola*
		4	5	T	48.4	*Desmognathus aeneus*
		4	5	T	156.6	*Desmognathus quadramaculatus*
		4	5	T	134.0	*Desmognathus brimleyorum*
		4	4	T	104.2	*Batrachoseps wrightorum*
		4	4	T	106.2	*Batrachoseps attenuatus*
		4	4	T	123.5	*Batrachoseps major*
		4	5	T	66.1	*Cryptotriton nasalis*
		4	5	T	60.4	*Dendrotriton xolocalcae*
		4	5	T	102.0	*Nyctanolis pernix*
		4	5	T	59.7	*Nototriton picadoi*
		4	5	T	74.5	*Bradytriton silus*
		4	5	T	51.1	*Oedipina complex*
		4	5	T	79.4	*Oedipina parvipes*
		4	5	T	156.3	*Oedipina cyclocauda*
		4	5	T	182.5	*Oedipina uniformis*
		4	5	T	51.9	*Thorius pennatulus*
		4	5	T	43.7	*Thorius pulmonaris*
		4	5	T	37.6	*Thorius macdougalli*
		4	5	T	45.7	*Chiropterotriton chondrostega*
		4	5	T	58.4	*Chiropterotriton multidentatus*
		4	5	T	96.6	*Chiropterotriton priscus*
		4	5	T	47.1	*Chiropterotriton dimidiatus*
		4	5	T	66.5	*Chiropterotriton lavae*
		4	5	T	125.6	*Pseudoeurycea bellii*
		4	5	T	80.6	*Pseudoeurycea cephalica*
		4	5	T	83.0	*Pseudoeurycea galeanae*
		4	5	T	39.1	*Parvimolge townsendi*
		4	5	T	105.4	*Pseudoeurycea leprosa*
		4	5	T	87.8	*Lineatriton orchimelas*
		4	5	T	98.3	*Pseudoeurycea werleri*
		4	5	T	51.7	*Pseudoeurycea rex*
		4	5	T	48.1	*Pseudoeurycea gadovii*
		4	5	T	72.0	*Pseudoeurycea robertsi*
		4	5	T	109.9	*Bolitoglossa subpalmata*
		4	5	T	83.7	*Bolitoglossa adspersa*
		4	5	T	75.5	*Bolitoglossa palmata*
		4	5	T	161.0	*Bolitoglossa dofleini*
		4	5	T	90.8	*Bolitoglossa morio*
		4	5	T	87.0	*Bolitoglossa engelhardti*
		4	5	T	81.9	*Bolitoglossa rostrata*
		4	5	T	61.9	*Bolitoglossa occidentalis*
		4	5	T	63.4	*Bolitoglossa rufescens*
		4	5	T	163.6	*Bolitoglossa platydactyla*
		4	5	T	103.8	*Bolitoglossa yucatana*
		4	5	T	123.3	*Bolitoglossa mexicana*

Legend:

Digit numbers
reduced
manus pes

Total length (mm)
> 190

4 5 T 98.7 Species

Life history
T = transforming
P = paedomorphic
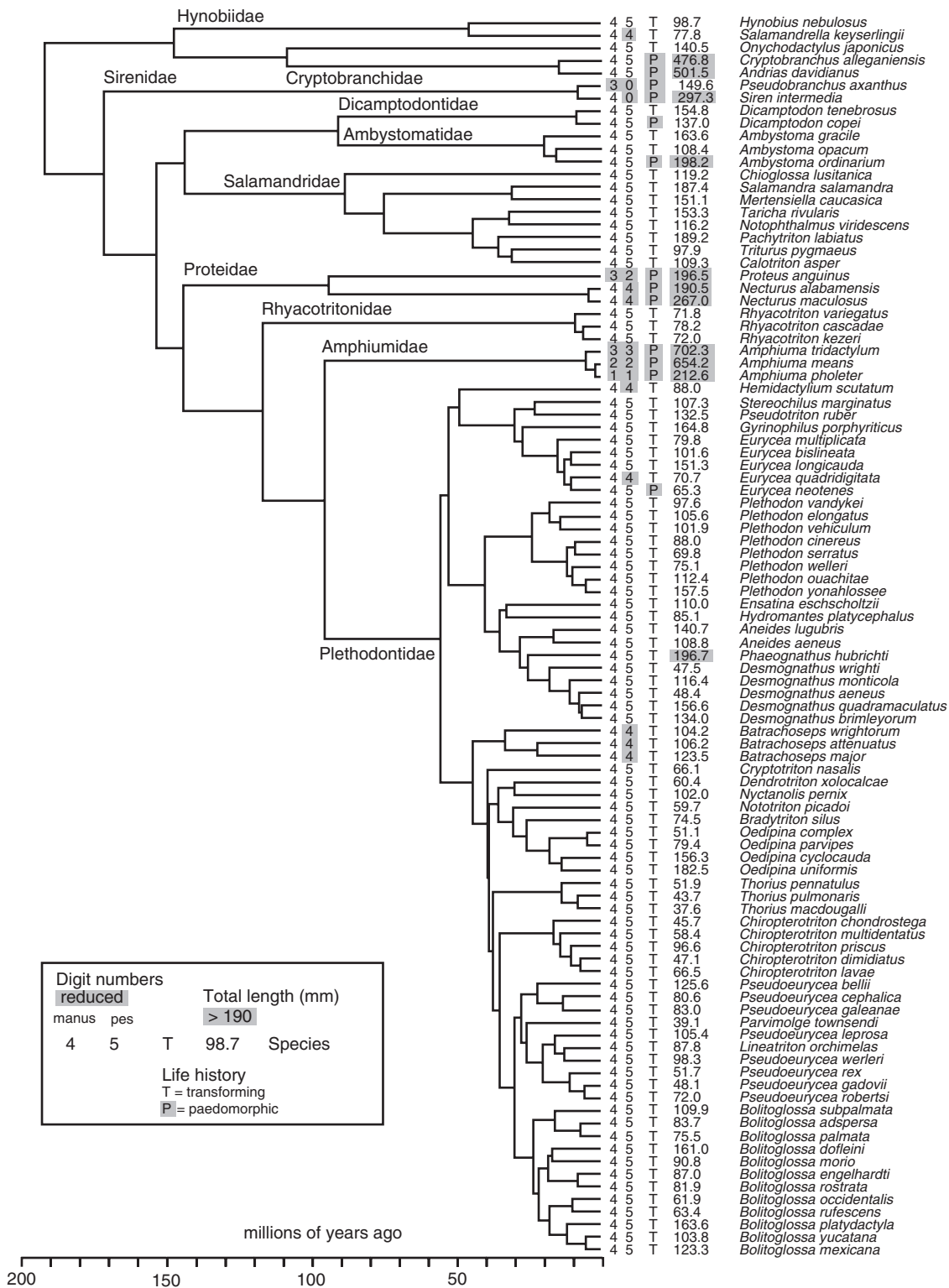
millions of years ago

200 150 100 50

Fig. 1. Summary of phylogeny and major patterns of variation in digit number (manus, pes), life history (transforming vs. paedomorphic), and body size (mean total length in mm) among salamanders. Gray shading indicates reduced digit numbers (relative to the primitive four fingers and five toes), paedomorphic species (those failing to complete metamorphosis), and species with unusually large body size (>190 mm; an arbitrary cut-off that was not used in any statistical analysis). Branch lengths indicate the estimated ages of clades from penalized likelihood analyses (from Wiens 2007; Wiens et al. 2007).

(2006). We used the phylogeny and branch lengths described above.

Using PGLS, we also tested for a relationship between paedomorphosis and evolutionary change in body size. For these analyses, we used PC1 as a measure of body size, but other measures gave similar results (J. J. Wiens, unpublished). We also tested whether paedomorphosis is associated with relative limb length (limb length/head length) or body elongation (total length/head length).

We considered those taxa that do not complete metamorphosis as being paedomorphic. Among the taxa we sampled, this included all amphiumids, cryptobranchids, proteids, and sirenids, as well as *Ambystoma ordinarium*, *Dicamptodon copei*, and *Eurycea neotenes*. We acknowledge that there is considerable controversy in the definition of paedomorphosis (e.g., Reilly et al. 1997), that generally it is only particular traits that can justifiably be considered paedomorphic rather than taxa, and that even these paedomorphic species differ considerably in their proportion of larval traits (e.g., Rose 1999). Furthermore, in the last three species listed (those in *Ambystoma, Dicamptodon, Eurycea*), some individuals do occasionally transform (e.g., Petranka 1998; Weisrock et al. 2006a). Nevertheless, we think that there is broad consensus among amphibian biologists that these families and species fail to complete normal metamorphosis and can reasonably be considered paedomorphic in their overall life history mode (e.g., Duellman and Trueb 1986; Petranka 1998; Rose 1999; Pough et al. 2004). We note that some direct developing salamanders have also been considered paedomorphic by some authors based on their small body size and on other seemingly juvenile skeletal traits (e.g., Alberch and Alberch 1981; Hanken and Wake 1993). However, we think that defining taxa as paedomorphic based on body size is problematic for our purposes here (given that we test for a relationship between paedomorphosis

and size), and it would be difficult to develop criteria based on other characters that could be used consistently across salamanders. Thus, although we acknowledge that our definition is not perfect (e.g., it excludes some taxa considered paedomorphic by other authors, including Alberch and Gale 1985), we think it is the most practical one that we can apply, given that there is broad agreement that the taxa that we define as paedomorphic are paedomorphic.

For comparison, many of the relationships were also tested using analyses of the raw data (i.e., not correcting for phylogeny). These analyses were conducted on the same 98 species used in the phylogenetic analyses. Although these analyses are presented for comparison to the phylogeny-based results, they should be interpreted with considerable caution because of the nonindependence of the data points due to phylogeny (Felsenstein 1985).

RESULTS

Major patterns of variation in digit number, life history, and body size are summarized in Fig. 1. Our results show that loss of digits is not associated with any particular body size, but is instead associated with evolutionary changes in body size (Fig. 2). Analyzing the raw data (without correcting for phylogeny, using the same 98 taxa), the relationship between body size and digit numbers is weak or nonsignificant, for both fingers ($r = 0.110$, $P = 0.2816$) and toes ($r = 0.068$, $P = 0.5048$). At the same time, there is a significant relationship between evolutionary changes in body size and changes in digit number based on independent contrasts, for both fingers ($r = 0.670$; $P < 0.0001$) and toes ($r = 0.576$, $P < 0.0001$).
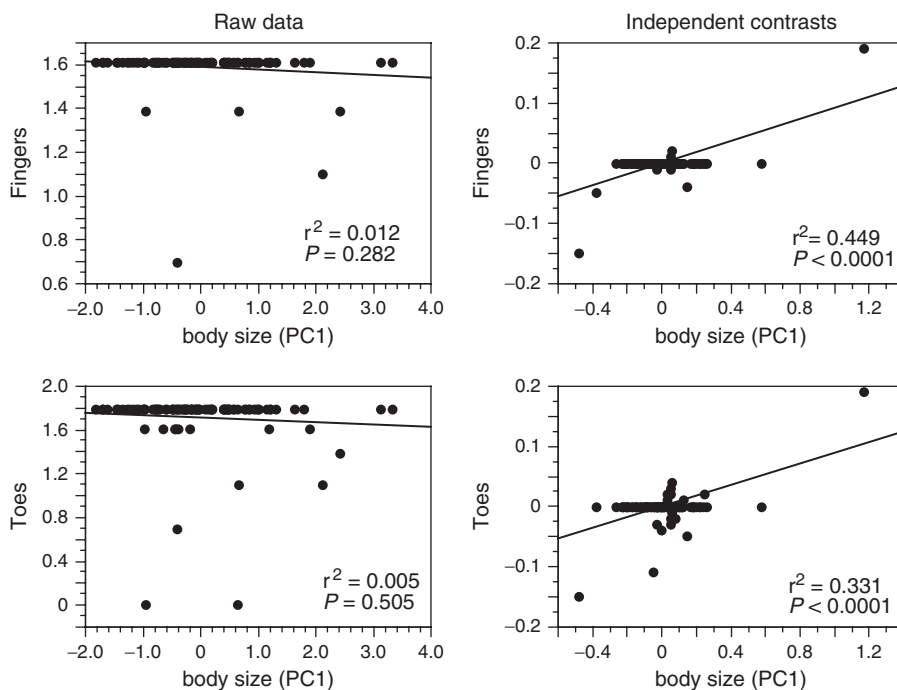

Fig. 2. Relationship between body size (scores on first principal component; PC1) and numbers of fingers and toes, using both raw data (ln-transformed) and independent contrasts. The results suggest that reductions in digit number are not associated with absolute body size, but with evolutionary changes in body size (primarily in *Amphiuma*).

The latter relationships are influenced very strongly by the genus *Amphiuma*, which contains the most extreme digit reduction, striking variation in body size, and relatively short branch lengths (Fig. 1). When *Amphiuma* is removed, these latter relationships are no longer significant. Thus, there is no evidence for an influence of body size reduction on the loss of the fifth toe (the most frequent change in digit number in salamanders; Fig. 1).

Using PGLS, there is a significant association between paedomorphosis and the loss of fingers ($r = 0.41$; $P < 0.01$) and toes ($r = 0.83$; $P < 0.01$). There is also a significant association between body size and paedomorphosis using PGLS ($r = 0.37$, $P < 0.01$). The relationship between paedomorphosis and increased body size is also evident from the raw data (Fig. 1). For example, the mean total body length of the paedomorphic species is 321.54 mm (range = 65.27–702.30), whereas the mean for transforming species is only 101.62 (37.56–196.74). Similarly, the mean body width is 20.51 mm for paedomorphic species (range = 3.22–49.92) and only 5.863 (1.88–21.03) for transforming species.

Analyses using independent contrasts show no relationship between loss of fingers and relative forelimb length ($r = 0.093$; $P = 0.3619$) but a significant relationship between loss of toes and relative hindlimb length ($r = 0.464$; $P < 0.0001$); similar results are found using ratios (forelimb/head length vs. fingers; $r = 0.162$; $P = 0.1111$; hindlimb/head length vs. toes; $r = 0.330$; $P = 0.0009$). There is a significant relationship in the raw data, using digit number and limb length divided by head length (finger vs. forelimb; $r = 0.507$; $P < 0.0001$; toe vs. hindlimb: $r = 0.673$; $P < 0.0001$); relationships are similar but weaker using ratios (forelimb/head length vs. fingers; $r = 0.221$; $P = 0.0284$; hindlimb/head length vs. toes; $r = 0.257$; $P < 0.0001$). There is a significant relationship between absolute limb size and digit number using independent contrasts (fingers vs. forelimb; $r = 0.667$; $P < 0.0001$; toes vs. hindlimb: $r = 0.709$; $P < 0.0001$), as might be expected from the analyses of overall body size (again, strongly influenced by *Amphiuma*). This relationship is much weaker in the raw data for fingers (finger vs. forelimb; $r = 0.221$; $P = 0.028$) but is similar for toes (toes vs. hindlimb; $r = 0.539$; $P < 0.0001$).

There is no relationship between body elongation and reduction in the length of the limbs using independent contrasts of residuals (relative total length vs. relative forelimb length: $r = 0.097$, $P = 0.3425$; relative total length vs. relative hindlimb length: $r = 0.079$, $P = 0.4413$), although the association is somewhat stronger using ratios (total/head vs. forelimb/head: $r = 0.052$, $P = 0.0235$; total/head vs. hindlimb/head: $r = 0.268$, $P = 0.0076$). Based on PGLS analyses, there does not appear to be any relationship between paedomorphosis and body elongation (paedomorphosis vs. total length/head length; $r = 0.11$, $P > 0.05$) or limb reduction (forelimb/head length: $r = 0.03$, $P > 0.05$; hindlimb/head length: $r = -0.09$, $P > 0.05$).

DISCUSSION

Correlates of digit loss in salamanders

Digit reduction is a widespread evolutionary trend in tetrapods, but why digit reduction occurs in a given lineage remains uncertain. Alberch and Gale (1985) suggested that in salamanders digit reduction was associated with either dwarfism or paedomorphosis. We found significant associations between digit loss and body size reduction and between digit loss and paedomorphosis. However, these trends are somewhat contradictory, given our finding that paedomorphic salamanders tend to be larger than transforming species. We found that digit reduction is only weakly associated with raw values of body size and is instead associated with evolutionary changes in body size in some lineages (particularly in *Amphiuma*).

Our results suggest that the relationship between digit reduction and body size in salamanders, if it is causal, may be context dependent. The most extreme cases of digit reduction in salamanders are associated with smaller species within clades of relatively large paedomorphic species (i.e., amphiumids, proteids, sirenids). In these cases, digits are lost in taxa that are clearly larger than other species that have retained all digits but that are outside these paedomorphic clades. Specifically, the most extreme digit reduction in amphiumids, sirenids, and proteids is seen in the relatively small species *Amphiuma pholeter*, *Pseudobranchus striatus*, and *Proteus anguinus* (respectively). Yet, there are many taxa that are considerably smaller but have not lost digits, including all rhyacotritonids, some salamandrids (e.g., some *Triturus*), and many species of diverse plethodontid genera (e.g., *Desmognathus aeneus, Plethodon cinereus, Thorius*).

What might explain this context dependence? Hanken and Wake (1993) proposed that the effects of miniaturization might be most severe in taxa with large genomes and correspondingly large cell sizes. This seems particularly relevant for digit reduction in salamanders. Alberch and Gale (1985) suggested that digits are lost when there is a limited number of cells in the developing limb bud, and there is considerable variation in genome sizes among salamanders (Duellman and Trueb 1986). Thus, the combination of a small limb size and large genome size might lead to digit reduction, even if the absolute size of the organism and limb are not unusually small. In order to address this hypothesis (if only crudely), we obtained data on genome sizes for 65 of the 98 species included here (from the Animal Genome Size Database; Gregory 2007; using C-value measurements based on Feulgen densitometry), and tested whether limb size divided by cell size was significantly related to digit number (with the expectation that species with large cell size and a small limb size would have extreme digit reduction), using independent contrasts of log-transformed variables as described above. Although we found significant relationships between limb/cell size and digit num-

ber for both the fore and hindlimb (fore: $r = 0.348$; $P = 0.0045$; hind: $r = 0.330$; $P = 0.0073$), these relationships are basically the same as those between digit number and limb size alone when repeated for the same taxa (fore: $r = 0.370$; $P = 0.0024$; hind: $r = 0.324$; $P = 0.0084$). Thus, accounting for differences in genome size does little to explain the relationship between limb size and digit number. Indeed, many salamanders with large genome sizes and small absolute limb size show no digit reduction (e.g., *Lineatriton, Oedipina*), despite the arguments of Wake (1991) based on *Batrachoseps* and *Parvimolge*.

Based on our results, the most important context for size-associated digit loss involves a paedomorphic life-history strategy. Thus, digit reduction might occur through the combination of a relatively small limb size in paedomorphic lineages with a relatively slow developmental rates (this possibility was not actually suggested by Alberch and Gale [1985] but is fully consistent with their arguments). Unfortunately, this hypothesis is difficult to refute or support with the morphological and developmental data at hand. For example, it is unclear if the paedomorphic lineages in question actually have slower developmental rates, and particularly at the developmental time and anatomical location relevant to the determination of digit number.

We found the relationship between digit loss and the evolution of a snake-like body form in salamanders to be somewhat ambiguous, in contrast to findings from squamate reptiles (e.g., Lande 1978; Greer 1991; Greer et al. 1998; Wiens and Slingluff 2001; Wiens et al. 2006). We found a significant relationship between relative limb size and digit reduction only for the raw data and for the independent contrast analyses for toes. Similarly, a relationship between limb-size reduction and body elongation was not consistently supported. This ambiguity is somewhat surprising in that many of the species with extreme digit reduction have relatively elongate bodies and relatively small limbs (e.g., amphiumids, sirenids). However, there are some exceptions. For example, the sirenid genus *Siren* retains the full complement of digits on the forelimb. Furthermore, even within these two families, many shifts in digit number seem to be associated with changes in body size in taxa with similar body shape (loss of fingers in *Pseudobranchus*, changes in digit number within *Amphiuma*). Although the proteid genus *Proteus* has a relatively elongate body and extreme digit reduction, the relative limb length is no more reduced than in some *Necturus*. Furthermore, many taxa have lost one toe but lack extreme body elongation or limb reduction (e.g., *Eurycea quadradigitata, Hemidactylium scutatum, Salamandrella keyserlingii*). Finally, there are other salamanders that have elongate bodies and relatively small limbs but exhibit no digit loss (e.g., *Lineatriton, Oedipina*). Our results show that the relationships between digit reduction, limb reduction, and body elongation that appear to be very strong in squamate reptiles (e.g., Wiens and Slingluff 2001) are considerably more ambiguous in salamanders.

Astute readers will notice that we have said nothing about the possible functional consequences or selective causes of digit loss in salamanders. Alberch and Gale (1985) argued that there was no selection for the loss of digits per se, and that these losses occurred as a consequence of changes in size or developmental rates. The most common change in digit number in salamanders is the loss of the fifth toe (Fig. 1) and we found little evidence to suggest that this loss is related to body size or paedomorphosis. But as noted by Alberch and Gale (1985) four-toed species differ dramatically in habitat (i.e., aquatic to terrestrial-fossorial), body size (Fig. 1), and body proportions, and so the functional significance of these losses is not obvious. The most extreme digit reduction in salamanders occurs in species that are paedomorphic, relatively elongate, and aquatic (i.e., *Amphiuma, Proteus, Pseudobranchus*). We speculate that extreme digit reduction might be facilitated by the presumably reduced importance of the limbs and digits in body support and locomotion in the aquatic environment. Thus, the relationship between aquatic habitat use and paedomorphosis may help explain the relationship between paedomorphosis and digit reduction. However, much digital reduction occurs within these aquatic, paedomorphic lineages, suggesting that merely being aquatic and paedomorphic is not sufficient to explain extreme digit loss.

Paedomorphosis and body size

Our results show that paedomorphosis is associated with increased body size in salamanders. All of the consistently non-transforming clades (i.e., amphiumids, cryptobranchids, proteids, sirenids) have relatively large body sizes relative to other salamanders. In contrast, paedomorphic species in clades that are not consistently pedomorphic have body sizes similar to their congeners, as in *Ambystoma, Dicamptodon,* and *Eurycea*. We are not aware of any studies in other organisms in which an association between increased body size and paedomorphosis has been explicitly documented. However, we suspect that many other cases exist that have yet to be analyzed quantitatively. For example, leatherback sea turtles (Dermochelyidae; *Dermochelys*) have very poorly ossified skeletons (seemingly a paedomorphic trait), yet are the largest extant turtles (Pough et al. 2004).

We find this relationship to be surprising for at least three reasons. First, paedomorphosis by definition involves a slowing or truncation of ontogeny in one or more traits (Alberch et al. 1979), whereas an increase in body size presumably involves an acceleration or extension of ontogeny for many parts of the body. Second, many studies have documented relationships between reduced body size and other paedomorphic traits (e.g., Alberch and Alberch 1981; Yeh 2002). Third, although individual taxa may possess a mixture of paedomorphic and peramorphic traits, the effects of paedomorphosis (in terms of failure to undergo metamorphosis) in

salamanders are dramatic and involve many different aspects of the morphology (e.g., external morphology, hyobranchial apparatus, skull elements; Rose 1999). The proportion of larval traits does vary among salamanders, and the largest paedomorphic taxa (the cryptobranchids *Andrias* and *Cryptobranchus*) clearly have a mixture of adult and larval traits (Duellman and Trueb 1986; [28]Rose 1999). Nevertheless, the two paedomorphic clades with the most larval traits (e.g., sirenids, proteids; Rose 1999) also have relatively large body size.

Although we find this relationship between increased body size and paedomorphosis to be surprising, there are other examples where body size and the developmental trajectories of other traits are similarly discordant. For example, even though one might expect miniaturized taxa to have generally reduced ossification, Hanken (1982) documented uniquely increased ossification of some limb skeletal elements in minute *Thorius* salamanders. Similarly, Trueb and Alberch (1985) noted increases in some aspects of skull ossification in miniaturized anurans of the genus *Brachycephalus*.

Why do paedomorphic salamanders tend to be larger than their transforming relatives? Some of the important theory on the evolution of amphibian life histories suggests that larval growth and the timing of metamorphosis are intimately related (e.g., Wilbur and Collins 1973; Werner 1986; Rowe and Ludwig 1991). Based on this theoretical work, individuals may delay metamorphosis in favor of increased larval growth, a trend that might lead to species that lack metamorphosis entirely and have very large body sizes, as in amphiumids, cryptobranchids, proteids, and sirenids. More proximally, metamorphosis in amphibians appears to be regulated by thyroid hormone (TH), and paedomorphosis may be related to reduction in levels of TH, or to losses of tissue sensitivity to TH (Rose 1999). Some studies have found that amphibian larvae treated with prolactin (an antagonist to TH) grow rapidly and delay metamorphosis (e.g., Etkin and Gona 1967). In general, the link between increased body size and paedomorphosis in salamanders could be an interesting area for future ecological and developmental research, although it may be complicated somewhat by the phylogenetic distance between the paedomorphic families and their closest relatives.

CONCLUSIONS

In this paper, we have examined the factors that may be correlated with loss of digits in salamanders. Our results suggest that these losses are associated with both paedomorphosis and with evolutionary reductions in body size (but not with absolute body size). We also find that paedomorphic (nontransforming) salamanders tend to be larger than other salamanders, and ours may be the first study to explicitly document such a trend.

Although further study will be needed to elucidate the developmental mechanisms that underlie these trends, our results offer two general lessons. First, they suggest that the effects of body size on morphological variation may be extremely context dependent, and that the consequences of body size may vary from clade to clade within a larger group (see also Yeh 2002). Second, they further demonstrate that body size and overall somatic development (i.e., whether a species is paedomorphic or undergoes metamorphosis) may be decoupled and vary in opposing directions.

REFERENCES

Alberch, P., and Alberch, J. 1981. Heterochronic mechanisms of morphological diversification and evolutionary change in the Neotropical salamander, *Bolitoglossa occidentalis* (Amphibia: Plethodontidae). *J. Morph.* 167: 249–264.

Alberch, P., and Gale, E. 1985. A developmental analysis of an evolutionary trend: digital reduction in amphibians. *Evolution* 39: 8–23.

Alberch, P., Gould, S. J., Oster, G. F., and Wake, D. B. 1979. Size and shape in ontogeny and phylogeny. *Paleobiology* 5: 296–317.

AmphibiaWeb: Information on amphibian biology and conservation. [web application]. Berkeley, California: AmphibiaWeb. Available: http://amphibiaweb.org/. (Accessed: November 2007).

Caputo, V., Lanza, B., and Palmieri, R. 1995. Body elongation and limb reduction in the genus *Chalcides* Laurenti 1768 (Squamata Scincidae): a comparative study. *Trop. Zool.* 8: 95–152.

Carroll, R. L. 1988. *Vertebrate Paleontology and Evolution*. W. H. Freeman, New York.

Crumly, C. R., and Sánchez-Villagra, M. 2004. Patterns of variation in the phalangeal formulae of land tortoises Testudinidae: developmental constraint imposed by size and phylogenetic history. *J. Exp. Zool. Mol. Dev. Evol.* 302B: 134–146.

Duellman, W. E., and Trueb, L. 1986. *Biology of Amphibians*. McGraw-Hill, New York.

Etkin, W., and Gona, A. G. 1967. Antagonism between prolactin and thyroid hormone in amphibian development. *J. Exp. Zool.* 165: 249–258.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125: 1–15.

Futuyma, D. J. 2005. *Evolution*. Sinauer, Sunderland, MA.

Galis, F., van Alphen, J. J. M., and Metz, J. A. J. 2001. Why five fingers? Evolutionary constraints on digit number. *Trends Ecol. Evol.* 16: 637–646.

Greer, A. E. 1991. Limb reduction in squamates: identification of the lineages and discussion of the trends. *J. Herpetol.* 25: 166–173.

Greer, A. E., Caputo, V., Lanza, B., and Palmieri, R. 1998. Observations on limb reduction in the scincid lizard genus *Chalcides*. *J. Herpetol.* 32: 244–252.

Gregory, T. R. 2007 Animal Genome Size Database. http://www.genomesize.com.

Hanken, J. 1982. Appendicular skeletal morphology in minute salamanders, genus *Thorius* (Amphibia: Plethodontidae): growth regulation, adult size determination, and natural variation. *J. Morphol.* 174: 57–77.

Hanken, J. 1983. Genetic variation in a dwarfed lineage, the Mexican salamander genus *Thorius* (Amphibia: Plethodontidae): taxonomic, ecologic, and evolutionary implications. *Copeia* 1983: 1051–1073.

Hanken, J., and Wake, D. B. 1993. Miniaturization of body size: organismal consequences and evolutionary significance. *Ann. Rev. Ecol. Syst.* 24: 501–519.

Kohlsdorf, T., and Wagner, G. P. 2006. Evidence for the reversibility of digit loss: a phylogenetic study of limb evolution in *Bachia* (Gymnophthalmidae: Squamata). *Evolution* 60: 1896–1912.

Lande, R. 1978. Evolutionary mechanisms of limb loss in tetrapods. *Evolution* 32: 73–92.

Larson, A., and Dimmick, W. W. 1993. Phylogenetic relationships of the salamander families: an analysis of congruence among morphological and molecular characters. *Herpetol. Monogr.* 7: 77–93.

Martins, E. P. 2004. COMPARE, version 4.6b. Computer programs for the statistical analysis of comparative data. Distributed by the author at http://compare.bio.indiana.edu/. Department of Biology, Indiana University, Bloomington, IN.

Martins, E. P., and Hansen, T. F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into analysis of interspecific data. *Am. Nat.* 149: 646–667.

Ord, T. J., and Martins, E. P. 2006. Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behavior. *Anim. Behav.* 71: 1411–1429.

Petranka, J. W. 1998. *Salamanders of the United States and Canada.* Smithsonian Inst. Press, Washington, DC.

Pough, F. H., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitzky, A. H., and Wells, K. D. 2004. *Herpetology.* 3rd Ed. Pearson Prentice Hall, Upper Saddle River, NJ.

Reilly, S. M., Wiley, E. O., and Meinhardt, D. J. 1997. An integrative approach to heterochrony: distinguishing intraspecific and interspecific phenomena. *Biol. J. Linn. Soc.* 60: 119–143.

Rohlf, F. J., and Sokal, R. R. 1995. *Statistical Tables.* W. H. Freeman and Company, New York, NY.

Rose, C. S. 1999. Hormonal control in larval development and evolution–amphibians. In B. K. Hall and M. H. Wake (eds.). *The Origin and Evolution of Larval Forms.* Academic Press, San Diego, CA, pp. 167–217.

Rowe, L., and Ludwig, D. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology* 72: 413–427.

Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* 19: 101–109.

Shapiro, M. D., Hanken, J., and Rosenthal, N. 2003. Developmental basis of evolutionary digit loss in the Australian lizard *Hemiergis. J. Exp. Zool. B Mol. Devel. Evol.* 297B: 48–56.

Shubin, N., Tabin, C., and Carroll, S. 1997. Fossils, genes, and the evolution of animal limbs. *Nature* 388: 639–648.

Stopper, G. F., and Wagner, G. P. 2005. Of chicken wings and frog legs: a smorgasbord of evolutionary variation in mechanisms of tetrapod limb development. *Dev. Bio.* 288: 21–39.

Stopper, G. F., and Wagner, G. P. 2006. Inhibition of *Sonic hedgehog* signaling leads to posterior digit loss in *Ambystoma mexicanum*: parallels to natural digit reduction in urodeles. *Dev. Dyn.* 236: 321–331.

Trueb, L., and Alberch, P. 1985. Miniaturization and the anuran skull: a case study of heterochrony. In H. R. Duncker and G. Fleischer (eds.). *Functional Morphology of the Vertebrates.* Gustav Fischer Verlag, New York, pp. 113–121.

Wake, D. B. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? *Am. Nat.* 138: 543–567.

Weisrock, D. W., Shaffer, H. B., Storz, B. L., Storz, S. R., and Voss, S. R. 2006a. Multiple nuclear gene sequences identify phylogenetic species boundaries in the rapidly radiating clade of Mexican ambystomatid salamanders. *Mol. Ecol.* 15: 2489–2503.

Weisrock, D. W., et al. 2006b. A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata). *Mol. Phylogenet. Evol.* 41: 368–383.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.* 128: 319–341.

Wiens, J. J. 2007. Global patterns of species richness and diversification in amphibians. *Am. Nat.* 170: S86–S106.

Wiens, J. J., and Slingluff, J. L. 2001. How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. *Evolution* 55: 2303–2318.

Wiens, J. J., Bonett, R. M., and Chippindale, P. T. 2005. Ontogeny discombobulates phylogeny: paedomorphosis and higher-level salamander phylogeny. *Syst. Biol.* 54: 91–110.

Wiens, J. J., Brandley, M. C., and Reeder, T. W. 2006. Why does a trait evolve multiple times within a clade? Repeated evolution of snake-like body form in squamate reptiles. *Evolution* 60: 123–141.

Wiens, J. J., Parra-Olea, G., Garcia-Paris, M., and Wake, D. B. 2007. Phylogenetic history underlies elevational patterns of biodiversity in tropical salamanders. *Proc. R. Soc. Lond. B* 274: 919–928.

Wilbur, H. M., and Collins, J. P. 1973. Ecological aspects of amphibian metamorphosis. *Science* 182: 1305–1314.

Yeh, J. 2002. The effect of miniaturized body size on skeletal morphology in frogs. *Evolution* 56: 628–641.

APPENDIX A1. SPECIMENS EXAMINED.

Ambystomatidae

Ambystoma annulatum: CM 50001, 54032, 54033, 54050, 54050c, 54050e, 54050b, 130388, 130390. *Ambystoma barbouri:* CM 43531, 43532, 52791. *Ambystoma californiense*: CM 51565. *Ambystoma cingulatum:* CM 29138–39. *Ambystoma gracile*: CM 7151–52, 62254. *Ambystoma jeffersonianum:* CM 9341, 11797, 14346, 14347, 13840, 26649, 26651, 40905, 43835, 117269. *Ambystoma laterale:* CM 70372. *Ambystoma mabeei:* CM 39670, 129528, 129250, 129252, 129265, 129265. *Ambystoma macrodactylum:* CM 32774, 32774b, 32774c, 32774d, 32774e, 35644, 38832, 38831. *Ambystoma maculatum:* CM 47431, 47404, 47410, 47414, 47419, 54028, 54029, 54030, 54031. *Ambystoma opacum:* CM 18635, 120114, 120337, 120334, 120341, 120347, 120349, 127275, 146643, 146642. *A. ordinarium:* CM 45908, 45911, 45913, 45914. *Ambystoma talpoideum:* CM 27571, 28976, 28977, 38978. *Ambystoma texanum*: CM 39955, 47435, 47436, 47439, 47446, 52722, 52722b, 52722d, 52722e, 52722 h. *Ambystoma tigrinum mavortium*: CM 39328, 42419, 60335, 60337, 60340, 60341, 130586, 130587, 130589, 130591. *Ambystoma velasci:* CM 45902, 45903, 45904, 45905, 45906

Amphiumidae

Amphiuma means: CM 5156, 18812, 21433b, 21433i, 125958, 128107. *Amphiumal pholeter*: UF 28810, 28811, 28812, 28813. *Amphiuma tridactylum*: CM 7584, 19495, 25755, 31902, 31925, 41664, 44852

Cryptobranchidae

Andrias davidianus: CM 56449. *Cryptobranchus alleganiensis*: CM 3746, 3747, 21651, 36604, 37480w, 112202, 113385, 143510

Dicamptodontidae

Dicamptodon copei: MVZ 192671, 192677. *Dicamptodon ensatus*: CM 62286, 62288; MVZ 72663, 72664. *Dicamptodon tenebrosus*: CM 40025

Hynobiidae

Batrachuperus pinchonii: CAS 194450, 194453, 194454, 194460. *Batrachuperus yenyuanensis*: CAS 194489, 194492, 194497, 194500. *Hynobius abei*: CAS 14155, 14156, 14157. *Hynobius dunni*: CM 68226–27. *Hynobius leechii*: CAS 31886, 31906, 32047, 32101. *Hynobius lichenatus*: CM 68230–31. *Hynobius naevius*: CM 68232. *Hynobius nebulosus*: CAS 26169, 26254, 26298, 26326. *Hynoboius nigrescens*: CM 60643, 68234. *Hynobius stejnegeri*: CM 5806. *Hynobius tsuensis*: CAS 26390, 26415, 26464; CM 5804, 68236. *Liua shihi*: CM 93748–49. *Onychodactylus japonicus*: CM 60644, 68238. *Pachyhynobius shangchengensis*: CAS 194233–36. *Paradactylodon mustersi*: CAS 147040, 147077, 147086. *Ranodon sibiricus*: CAS 199692, 199693, 199694, 199695. *Salamandrella keyserlingii*: CM 47583–84

Plethodontidae

Aneides aeneus: CM 62343, 134905, 134906, 134923, 134924, 134942, 134954, 134955, 134962, 134963. *Aneides flavipunctatus*: CM 51576, 51577, 51578, 51579, 51580. *Aneides hardii*: CM 72318, 72353, 72357, 72383, 72384. *Aneides lugubris*: CM 24402, 33897, 38821, 62356, 62359, 62360, 62361. *Aneides vagrans*: CM 40020, 40021, 45123, 45124, 45127. *Batrachoseps attenuatus*: CM 6207z, 10961, 25980, 25981, 25982, 25991, 25994, 23496, 33889, 62368. *Batrachoseps major*: CM 9268, 62371, 62373, 135016, 135017. *Batrachoseps wrightorum*: CM 32801. *Bolitoglossa adspersa*: CM 62374. *Bolitoglossa dofleini*: CM 57646, 57647, 57648, 57657. *Bolitoglossa engelhardti*: CM 9305–06. *Bolitoglossa mexicana*: CM 105764. *Bolitoglossa morio*: CM 42030, 42045, 42064, 42122, 42247. *Bolitoglossa mulleri*: CM 57660–63. *Bolitoglossa occidentalis*: CM 93730, 93732. *Bolitoglossa palmata*: CM 13118. *Bolitoglossa platydactyla*: CM 40651, 43556, 43557. *Bolitoglossa robusta*: CM 107404–05. *Bolitoglossa rostrata*: CM 39741, 135713, 135714, 135715. *Bolitoglossa rufescens*: CM 57698, 57702e, 57703f, 57703o, 57715t. *Bolitoglossa salvini*: CM 17399. *Bolitoglossa subpalmata*: CM 62377, 62414, 62418, 62425. *Bolitoglossa yucatana*: CM 45224. *Bradytriton silus*: LACM 134566. *Chiropterotriton chiropterus*: CM 57777, 57777c, 57777d, 57777 g, 57777e. *Chiropterotriton chondrostega*: CM 39985–86. *Chiropterotriton dimidiatus*: CM 39984. *Chiropterotriton lavae*: CM 39983. *Chiropterotriton multidentatus*: CM 39995–96. *Chiropterotriton priscus*: CM 42502, 42517, 42522, 42524, 42533, 42449, 42458, 42464, 42483, 42496. *Cryptotriton nasalis*: CM 9307. *Dendrotriton xolocalcae*: CM 39987–88. *Desmognathus aeneus*: CM 46269, 46271, 56325b, 56326, 130637. *Desmognathus auriculatus*: CM 130913, 130916, 130917, 130931, 130932, 130933, 130936, 130942, 130947, 130987. *Desmognathus brimleyorum*: CM 19525, 74994, 74995, 74996, 130726, 130727, 130728,

131015, 131016. *Desmognathus fuscus*: CM 38669, 58841, 61948, 61949, 68604, 74829, 86250, 86478, 86536, 86265, 131652, 131681, 131806, 131831, 131832. *Desmognathus imitator*: CM 90888, 90889, 123445, 123451, 123466. *Desmognathus marmoratus*: CM 90903, 90906, 118135, 135671, 135672. *Desmognathus monticola*: CM 4181c, 4181 g, 4181 m, 30643s, 30643t, 30643ll, 30643pp, 30643rr, 30643ss, 138435. *Desmognathus ochrophaeus*: CM 5036, 5241, 5555, 5555b, 5555c, 5578 g, 5578c, 5749b, 5782b, 5873d. *Desmognathus quadramaculatus*: CM 127941, 127943, 128939, 128943, 128944, 134062, 134063, 134064, 134066, 134069. *Desmognathus welteri*: CM 127011, 127817, 127818, 127857, 128895, 128898, 128899, 128907, 128908, 128912. *Desmognathus wrighti*: CM 21588, 62515, 62516, 123550, 130686. *Ensatina eschscholtzii xanthopicta*: CM 55775–77. *Eurycea bislineata*: CM 19124, 20275, 23358, 27517, 27924, 29886, 29957, 32528, 34711. *Eurycea cirrigera*: CM 113527, 113988, 113997, 134532, 134575, 134582, 134592, 134594, 134613, 134618. *Eurycea junalaska*: CM 89536, 89537, 89538, 89539, 89540, 134471, 135487. *Eurycea latitans*: MVZ 121232, 121239, 121352, 121353. *Eurycea longicauda*: CM 12572, 12713, 19167, 24886, 29747, 29810, 29814, 30395, 31189, 34314. *Eurycea lucifuga*: CM 62532, 138893, 138895, 138896, 138899, 138900, 138901, 138902. *Eurycea multiplicata*: CM 10619, 25129, 25029, 50009, 50010, 50011, 50012, 50013, 62533. *Eurycea nana*: CM 134494–98. *Eurycea neotenes*: MVZ 119960, 119961, 119964, 120007, 120081, 120084. *Eurycea quadridigitata*: CM 9401, 9401c, 9401i, 9401j, 9401k, 9401n, 9521, 11003, 27127, 27128. *Eurycea rathbuni*: CM 21945. *Eurycea spelaea*: CM 11276. *Eurycea sosorum*: TNHC 51185–86. *Eurycea tridentifera*: MVZ 120565, 120566, 120578, 120583. *Eurycea tynerensis*: CM 42420a, 42420e, 42420 g, 42420 h, 42420j, 42420 m, 122339, 122340, 122341, 134998. *Eurycea wilderae*: CM 6542, 6345, 6345b, 6559b, 6559j, 6559k, 17696b, 17696d, 134525, 134527. *Gyrinophilus porphyriticus*: CM 6003, 10302, 10302f, 10302 g, 10302b, 12806, 14059, 14060, 19650,18453. *Haideotriton wallacei*: CM 72253, 72254. *Hemidactylium scutatum*: CM 5268, 29997, 30001, 30008, 30009, 30011 31275, 35160, 35301, 35302. *Hydromantes genei*: CM 4940. *Hydromantes italicus*: CM 4941, 9787-90. *Hydromantes platycephalus*: CM 34694, 35642, 38827. *Lineatriton orchimelas*: CM, 2 specimens. *Nototriton picadoi*: CM 57897, 62435, 62436. *Nyctanolis pernix*: MCZ-A 100154. *Oedipina complex*: CM 8331. *Oedipina cyclocauda*: CM 68241. *Oedipina parvipes*: CM 8332, 38829. *Oedipina uniformis*: CM 68242. *Parvimolge townsendi*: MCZ 24712, 24715, 24718; USNM 224653, 224656, 224657, 224658. *Phaeognathus hubrichti*: CM 39510, 90099, 135683, 135684, 135686. *Plethodon albagula*: CM 21946, 21947, 21954, 21956, 21957. *Plethodon angusticlavius*: CM 50014, 54037, 139303. *Plethodon caddoensis*: CM 62540. *Plethodon chlorobryonis*: CM 125976, 127013, 146627. *Plethodon cinereus*: CM 26977, 26977b, 26977c, 26977e, 26977I, 68657, 74172, 74188, 74234, 83037. *Plethodon cylindraceus*:

CM 126853, 127074, 127180, 127265, 127623, 127626, 127627, 127796, 127797, 127873. *Plethodon dorsalis:* CM 5853, 7609, 10939, 11431, 13586, 114330. *Plethodon dunni:* CM 32787, 32788, 32794, 32795, 135208. *Plethodon elongatus:* CM 7155-56. *Plethodon glutinosus:* CM 33710, 33710b, 33710c, 33710d, 33710e, 34348, 35004f, 35004 m, 35324, 36388. *Plethodon grobmani:* CM 9385, 21478l, 21478n, 26619, 135258, 135262, 135264, 135265, 135279. *Plethodon hoffmani:* CM 29067f, 29067j, 29067k, 29067s, 29067w, 32089, 32089c, 32089 g, 32089i, 46628c. *Plethodon hubrichti:* CM 92148, 127034, 127036, 127038, 127039, 127043, 127045, 127051, 127052, 127082. *Plethodon idahoensis:* CM 86857, 96958. *Plethodon jordani:* CM 15808, 17724, 17725, 17726, 135409, 135424, 135427, 135428, 143110. *Plethodon kentucki:* CM 135325, 127829, 127830, 127836, 127837, 129564. *Plethodon mississippi:* CM 135326, 135329, 135359, 135360, 135361, 135384, 135385, 135386, 135389, 135392. *Plethodon neomexicanus:* CM 72404 72406, 72411, 72420, 72422, 72433, 72439, 72442, 72443, 72460. *Plethodon nettingi:* CM 18730, 28505, 34437, 86023, 86382, 86385, 86399, 86402, 143133, 143134. *Plethodon ouachitae:* CM 62574, 75000. *Plethodon punctatus:* CM 120074, 127454, 127460, 127605, 127684, 127685, 127686, 127696, 127700, 127701, 127702. *Plethodon richmondi:* CM 24120c, 24120f, 24120j, 24120n, 24121b, 24125, 24129, 34575, 34576, 43851. *Plethodon serratus:* CM 50016, 50017, 143139. *Plethodon shenandoah:* CM 77470, 77471, 77474, 77485, 77494. *Plethodon teyahalee:* CM 135217, 135218, 135252, 135253. *Plethodon vandykei:* CM 58174b, 58174d, 58174f. *Plethodon vehiculum:* CM 8680, 8681, 58160. *Plethodon ventralis:* CM 26551, 26555, 26556, 26557, 26558, 26559, 41692, 135185, 135187, 135197. *Plethodon wehrlei:* CM 19600, 19600b, 19702, 62577, 62578, 62579, 62581, 130020, 130022, 139370. *Plethodon welleri:* CM 39704, 54752, 54752b, 135231, 135235, 135236, 135237, 135248, 123757, 123759. *Plethodon yonahlossee:* CM 17695, 17695b, 17695e, 17695 g, 119609, 119693, 120076, 120077, 135476, 135477, 135478, 143148. *Pseudoeurycea bellii:* CM 5807–09. *Pseudoeurycea cephalica:* CM 62590, 93723, 93734. *Pseudoeurycea gadovii:* CM 93735, 93737, 93738. *Pseudoeurycea galeanae:* CM 57886. *Pseudoeurycea leprosa:* CM 93722, 93725, 93727, 93729. *Pseudoeurycea rex*: CM 39742. *Pseudoeurycea robertsi:* CM 62592. *Pseudoeurycea werleri*: CM 46272, 135696, 135699, 135700, 135701. *Pseudotriton montanus*: CM 38809, 54455, 54458, 135530, 135531, 135532, 139402, 139403, 139404, 143149. *Pseudotriton ruber:* CM 113363, 113364, 116319, 118772, 118775, 122493, 143595, 143717, 144195, 144613. *Stereochilus marginatus:* CM 139662. *Thorius macdougalli:* CM 41268, 41269, 41270, 41486, 41487. *Thorius pennatulus:* CM 135732, 135735, 135736, 135744, 135746. *Thorius pulmonaris:* CM 68247.

Proteidae

Necturus alabamensis: UF 69263, 72110, 72111, 72112. *Necturus beyeri:* TU 3940, 3941, 21200, 29002; UF 67024.

Necturus maculosus: CM 25804, 30110, 30418, 41285, 75895, 75896, 137009. *Necturus punctatus:* NCSM 32191, 32212, 32214, 32218. *Proteus anguinus:* CM 21948.

Rhyacotritonidae

Rhyacotriton cascadae: CM 32798, 32798b, 32798c, 32798d, 32798e, 32798f, 32798 g, 32798 h, 32798i, 58176. *Rhyacotriton kezeri:* MVZ 197436, 197446. *Rhyacotriton olympicus:* CM 38817, 58173c, 135029; MVZ 185912, 185914. *Rhyacotriton variegatus:* CM 40026, 51569, 51570

Salamandridae

Calotriton asper: CM 117245, 117246, 117249. *Chioglossa lusitanica:* CM 60788–89. *Cynops ensicauda:* CM 45840, 45841, 62291, 62293, 62294. *Cynops pyrrhogaster:* CM 135757, 135763, 135764, 135765, 135767, 135768, 135769, 135771, 135775, 135787. *Echinotriton andersoni:* CM 135781–82. *Lissotriton boscai:* CM 14719, 14720, 14721, 14178. *Lissotriton helveticus:* CM 60808, 60817, 60819, 60821. *Lissotriton montandoni:* CM 4939, 7603. *Lissotriton vulgaris:* CM 28061, 28062, 28063, 28064, 62339, 62338, 62340, 62341, 62342. *Mertensiell caucasica:* CM 9311. *Mesotriton alpestris:* CM 7602, 60800, 60801, 60804, 60805, 60806, 62324, 62325. *Neururgus crocatus:* FMNH 19627, 19629; MCZ-A 24182. *Neururgus strauchii:* MCZ-A-99110, USNM 348636. *Notophthalmus meridionalis:* CM 25839, 62295, 62296. *Notophthalmus perstriatus:* CM 11006, 11008. *Notophthalmus viridescens:* CM 14220b, 17559, 23291, 126331, 126952, 126953, 126956, 126958, 126959, 126960. *Ommatotriton vittatus:* CM 62336. *Pachytriton brevipes:* CM 93753–54. *Paramesotriton caudopunctatus:* CM 93750. *Paramesotriton chinensis:* CM 93751–52. *Paramesotriton hongkongensis:* CM 145812–13. *Pleurodeles waltl:* CM 52081, 52158, 52572, 52573, 52650, 55266c, 55266e, 55266f. *Salamandra atra:* CM 8684, 62303, 62304. *Salamandra salamandra bejarae:* CM 52132, 52133, 52134, 52646, 54272, 54276, 54616, 55740, 55741. *Salamandrina terdigitata:* FMNH 84907, 84908, 84910, 84911; MCZ-A-115367, 115368; USNM 71513, 71514, 71515, 71517. *Taricha granulosa:* CM 32773, 32773b-d, 32773f, 38822, 38888, 62307. *Taricha rivularis:* CM 62315–16. *Taricha torosa:* CM 5624, 5624b, 5624c, 5624f, 10959, 51571, 51572, 55783, 55784, 135052. *Triturus cristatus:* CM 7576, 11037, 11038, 11039, 11040, 11041, 62328, 62330. *Triturus pygmaeus:* CM 52135, 52577, 53071, 54344, 55731x, 55731ff, 55731tt, 55731uu, 55731yy, 55753. *Tylototriton kweichowensis:* USNM 95520, 95521, 95522, 95523, 95524. *Tylototriton verrucosus:* FMNH 215848, 215849, 215480, 215491

Sirenidae

Pseudobranchus axanthus: CM 20131, 20131b, 20131c, 20131d, 20131e, 20131f, 20131 g, 20131 h, 20131i, 20131j. *Siren intermedia nettingi:* CM 49277, 49279, 49282, 49284, 49283. *Siren lacertina:* CM 18816, 21450, 21453, 21457, 21459

APPENDIX B

Table B1. Morphometric data. Values presented are within-species averages, N refers to the number of individuals per species

Species	N	Snout-vent length	Tail length	Head length	Snout-eye length	Body width	Forelimb length	Hindlimb length	Fingers	Toes
Ambystomatidae										
Ambystoma annulatum	9	84.889	76.422	12.433	7.122	9.456	17.211	20.789	4	5
Ambystoma barbouri	3	75.267	56.333	10.067	6.100	8.133	14.467	18.800	4	5
Ambystoma californiense	1	91.800	101.000	20.100	13.300	14.100	27.300	32.400	4	5
Ambystoma cingulatum	2	53.900	43.500	9.300	5.550	5.900	11.400	12.900	4	5
Ambystoma gracile	3	77.867	85.700	17.267	11.933	11.900	27.100	30.133	4	5
Ambystoma jeffersonianum	10	82.150	70.540	16.930	10.070	8.780	21.830	26.620	4	5
Ambystoma laterale	1	54.900	41.200	10.200	6.600	6.400	13.400	16.300	4	5
Ambystoma mabeei	6	66.100	44.817	11.083	6.750	7.850	15.200	18.950	4	5
Ambystoma macrodactylum	8	60.000	53.075	11.987	7.838	6.875	16.438	19.438	4	5
Ambystoma maculatum	9	81.378	72.833	15.911	9.489	10.433	18.467	21.344	4	5
Ambystoma opacum	10	65.560	42.880	12.610	8.570	9.340	14.840	18.740	4	5
Ambystoma ordinarium	4	110.850	87.300	27.100	16.175	20.850	34.250	35.650	4	5
Ambystoma talpoideum	4	63.500	44.775	13.875	9.175	10.700	17.600	24.000	4	5
Ambystoma texanum	10	71.850	55.970	10.610	6.440	8.940	14.750	18.240	4	5
Ambystoma tigrinum mavortium	10	96.610	81.570	20.490	13.040	16.420	28.090	32.880	4	5
Ambystoma velasci	5	84.520	74.200	18.800	10.700	13.380	25.660	25.780	4	5
Amphiumidae										
Amphiuma means	6	490.000	164.200	43.267	19.617	28.117	9.783	13.950	2	2
Amphiuma pholeter	4	164.750	47.900	12.075	5.725	6.525	2.025	1.850	1	1
Amphiuma tridactylum	7	557.714	144.586	51.786	24.300	32.743	13.543	19.929	3	3
Cryptobranchidae										
Andrias davidianus	1	331.000	170.500	74.800	32.900	48.300	53.000	66.100	4	5
Cryptobranchus alleganiensis	8	308.500	168.312	61.650	27.537	49.925	45.350	55.587	4	5
Dicamptodontidae										
Dicamptodon copei	2	83.250	53.800	14.100	8.850	11.400	19.100	20.800	4	5
Dicamptodon ensatus	4	108.000	70.800	25.325	16.500	16.975	27.000	35.100	4	5
Dicamptodon tenebrosus	1	91.600	63.200	22.400	15.000	14.900	24.900	31.700	4	5
Hynobiidae										
Batrachuperus pinchonii	4	105.150	99.750	28.150	11.125	15.300	24.650	30.600	4	4
Batrachuperus yenyuanensis	4	83.225	74.025	14.525	8.500	11.800	19.475	23.475	4	4
Hynobiu abei	3	57.767	28.100	9.633	6.100	8.067	12.867	15.867	4	5
Hynobius dunni	2	65.000	43.850	11.350	8.100	8.250	18.700	22.250	4	5
Hynobius leechii	4	51.650	29.200	8.225	5.675	7.075	10.725	13.000	4	5
Hynobius lichenatus	2	70.650	54.400	10.300	7.750	9.250	18.650	22.150	4	5
Hynobius naevius	1	74.100	50.200	12.300	8.400	9.900	16.500	17.500	4	5
Hynobius nebulosus	4	63.100	35.600	10.150	6.100	7.925	12.275	15.200	4	5
Hynobius nigrescens	2	67.250	47.500	12.150	8.050	9.100	19.900	23.100	4	5
Hynobius stejnegeri	1	61.200	40.200	11.200	7.100	6.900	14.200	17.500	4	5
Hynobius tsuensis	5	64.500	46.620	11.860	7.160	8.480	14.160	17.500	4	5
Liua shihi	2	74.500	72.500	15.850	9.300	10.450	19.450	23.950	4	5
Onychodactylus japonicus	2	64.500	76.000	10.100	7.800	7.000	16.350	19.550	4	5
Pachyhynobius shangchengensis	4	107.650	79.100	23.700	10.600	14.425	20.925	27.350	4	5
Paradactylodon mustersii	3	73.267	64.000	16.967	8.733	10.667	17.933	20.400	4	4
Ranodon sibiricus	4	85.975	97.050	19.475	9.975	11.625	22.950	27.250	4	5
Salamandrella keyserlingii	2	45.700	32.150	9.100	6.000	6.600	11.700	11.550	4	4
Plethodontidae										
Aneides aeneus	10	49.840	58.920	10.600	6.360	5.010	15.490	17.420	4	5
Aneides flavipunctatus	5	65.300	49.260	13.320	6.880	6.600	13.400	15.120	4	5
Aneides hardii	5	54.000	43.680	10.060	5.780	5.460	11.020	12.200	4	5
Aneides lugubris	7	79.314	61.414	17.100	9.657	10.700	20.357	23.157	4	5
Aneides vagrans	5	68.320	52.860	12.900	7.340	6.500	16.700	19.480	4	5

Table B1. (Contd.)

Species	N	Snout-vent length	Tail length	Head length	Snout-eye length	Body width	Forelimb length	Hindlimb length	Fingers	Toes
Batrachoseps attenuatus	10	44.600	61.650	6.090	3.610	3.240	5.830	6.280	4	4
Batrachoseps major	5	53.300	70.240	7.440	4.640	3.620	6.580	7.240	4	4
Batrachoseps wrightorum	1	44.600	59.600	7.700	4.500	3.200	8.000	8.700	4	4
Bolitoglossa adspersa	1	54.000	29.700	9.300	5.700	5.500	9.800	10.000	4	5
Bolitoglossa dofleini	4	103.350	57.625	18.300	11.600	15.500	22.025	23.250	4	5
Bolitoglossa engelhardti	2	45.750	41.200	9.550	6.150	4.200	11.050	11.650	4	5
Bolitoglossa mexicana	1	61.900	61.400	10.900	6.500	5.900	11.800	13.000	4	5
Bolitoglossa morio	5	51.880	38.920	9.460	6.420	6.380	11.840	12.560	4	5
Bolitoglossa mulleri	4	70.225	61.825	10.850	6.625	7.375	13.225	14.375	4	5
Bolitoglossa occidentalis	2	34.850	27.050	7.700	4.750	3.350	8.200	9.650	4	5
Bolitoglossa palmata	1	46.900	28.600	8.300	4.700	5.000	8.100	9.500	4	5
Bolitoglossa platydactyla	3	79.733	83.833	14.000	8.533	8.933	16.633	18.200	4	5
Bolitoglossa robusta	2	100.100	95.500	16.950	10.650	12.450	18.950	21.450	4	5
Bolitoglossa rostrata	4	44.725	37.175	8.350	5.575	4.250	9.350	9.975	4	5
Bolitoglossa rufescens	5	36.640	26.780	7.140	4.860	4.180	7.920	8.080	4	5
Bolitoglossa salvini	1	51.100	50.900	9.800	6.100	4.400	11.100	12.000	4	5
Bolitoglossa subpalmata	5	55.280	54.600	9.540	6.400	6.280	12.060	12.040	4	5
Bolitoglossa yucatana	1	56.900	46.900	9.500	6.600	5.700	10.800	12.400	4	5
Bradytriton silus	1	42.600	31.900	8.000	4.800	5.500	9.300	9.600	4	5
Chiropterotriton chiropterus	5	33.300	41.440	7.140	4.480	3.320	8.580	9.880	4	5
Chiropterotriton chondrostega	2	24.050	21.700	5.250	2.800	2.200	4.450	5.200	4	5
Chiropterotriton dimidiatus	1	25.100	22.000	5.100	2.700	2.100	4.200	5.300	4	5
Chiropterotriton lavae	1	30.000	36.500	6.900	4.400	2.600	8.700	8.800	4	5
Chiropterotriton multidentatus	2	29.600	28.800	6.500	3.650	2.400	6.600	7.900	4	5
Chiropterotriton priscus	10	47.970	48.670	8.900	5.300	5.110	9.850	11.370	4	5
Cryptotriton nasalis	1	29.600	36.500	6.200	3.700	2.700	5.900	6.200	4	5
Dendrotriton xolocalcae	2	32.950	27.500	7.250	4.300	2.550	7.950	9.250	4	5
Desmognathus aeneus	5	25.980	22.400	5.400	3.300	2.580	4.680	7.140	4	5
Desmognathus auriculatus	10	56.640	51.910	10.000	6.080	6.060	8.590	13.040	4	5
Desmognathus brimleyorum	9	74.956	59.044	14.222	8.744	8.989	13.300	19.044	4	5
Desmognathus fuscus	15	54.360	47.607	11.227	6.673	6.773	9.873	14.193	4	5
Desmognathus imitator	5	46.060	47.880	9.540	5.860	4.800	9.720	13.120	4	5
Desmognathus marmoratus	5	65.260	46.740	12.500	8.820	8.440	12.980	17.460	4	5
Desmognathus monticola	10	64.990	51.380	13.200	8.610	8.440	12.280	17.530	4	5
Desmognathus ochrophaeus	10	41.170	42.850	7.920	5.090	4.620	7.710	11.170	4	5
Desmognathus quadramaculatus	10	83.690	68.940	19.720	11.680	11.820	16.110	22.260	4	5
Desmognathus welteri	10	66.730	53.010	12.290	8.370	7.880	12.040	16.510	4	5
Desmognathus wrighti	5	29.120	18.360	5.960	3.520	2.720	6.000	7.680	4	5
Ensatina eschscholtzii xanthopicta	3	64.467	45.533	12.600	8.433	7.033	18.300	20.700	4	5
Eurycea bislineata	10	44.950	56.650	7.650	4.450	4.040	8.270	10.540	4	5
Eurycea cirrigera	10	41.610	58.140	7.940	4.450	3.770	9.240	11.500	4	5
Eurycea junalaska	7	43.514	42.943	8.600	4.729	4.329	9.929	11.843	4	5
Eurycea latitans	4	36.000	30.475	7.175	3.825	3.900	6.525	7.150	4	5
Eurycea longicauda	10	59.060	92.270	10.830	6.100	5.450	14.380	16.770	4	5
Eurycea lucifuga	9	62.867	93.711	13.444	8.756	6.378	18.600	20.467	4	5
Eurycea multiplicata	10	40.980	38.790	6.770	3.820	3.280	6.190	7.470	4	5
Eurycea nana	5	26.020	20.440	4.940	2.720	2.040	4.440	4.740	4	5
Eurycea neotenes	6	34.967	30.300	5.550	3.283	3.217	5.850	6.850	4	5
Eurycea quadridigitata	10	30.170	40.530	5.810	3.100	2.390	5.840	7.510	4	4
Eurycea rathbuni	1	54.300	48.300	12.000	7.500	5.900	19.200	19.100	4	5
Eurycea spelaea	1	50.700	50.700	10.200	5.900	5.400	11.200	12.600	4	5
Eurycea sosorum	2	25.400	17.550	5.000	2.550	2.250	5.400	5.800	4	5
Eurycea tridentifera	4	37.575	36.700	7.975	3.625	4.475	9.250	10.200	4	5
Eurycea tynerensis	10	33.250	27.420	5.420	3.080	2.480	4.650	5.080	4	5
Eurycea wilderae	10	39.320	50.620	7.050	3.880	3.570	8.040	9.740	4	5

Table B1. (Contd.)

Species	N	Snout-vent length	Tail length	Head length	Snout-eye length	Body width	Forelimb length	Hindlimb length	Fingers	Toes
Gyrinophilus porphyriticus	10	100.680	64.130	15.980	10.400	9.810	16.140	19.380	4	5
Haideotriton wallacei	2	25.450	15.600	5.000	3.100	1.500	6.150	6.550	4	5
Hemidactylium scutatum	10	40.230	47.720	7.400	4.290	4.180	8.210	9.220	4	4
Hydromantes genei	1	70.600	29.000	13.700	9.400	5.700	22.200	22.400	4	5
Hydromantes italicus	5	51.900	32.880	10.740	6.820	4.520	14.720	15.660	4	5
Hydromantes platycephalus	3	52.800	32.333	9.600	6.767	5.567	13.000	15.533	4	5
Lineatriton orchimelas	2	32.150	55.600	5.300	3.150	2.350	3.100	2.950	4	5
Nototriton picadoi	3	26.400	33.300	5.433	3.200	3.833	4.667	5.333	4	5
Nyctanolis pernix	1	39.900	62.100	8.500	5.300	3.500	11.800	13.900	4	5
Oedipina complex	1	31.400	19.700	5.300	2.900	2.000	5.300	5.400	4	5
Oedipina cyclocauda	1	49.300	107.000	7.000	3.600	3.300	5.500	5.900	4	5
Oedipina parvipes	2	39.750	39.600	6.400	3.450	2.850	6.050	8.100	4	5
Oedipina uniformis	1	49.800	132.700	7.000	3.500	3.300	4.500	4.000	4	5
Parvimolge townsendi	7	19.343	19.757	4.171	2.243	2.129	3.986	4.100	4	5
Phaeognathus hubrichti	5	107.980	88.760	14.680	9.240	8.900	9.800	12.200	4	5
Plethodon albagula	5	74.640	63.920	12.320	8.020	8.160	16.120	18.440	4	5
Plethodon angusticlavius	3	43.367	42.900	7.033	3.933	3.700	8.100	9.433	4	5
Plethodon caddoensis	1	38.000	45.300	8.200	5.000	3.600	9.200	10.900	4	5
Plethodon chlorobryonis	3	49.233	61.433	10.367	5.867	5.933	12.200	14.900	4	5
Plethodon cinereus	10	45.280	42.770	7.070	3.870	3.570	6.630	8.100	4	5
Plethodon cylindraceus	10	78.120	81.210	13.150	8.010	8.020	16.650	20.030	4	5
Plethodon dorsalis	6	40.933	30.933	6.700	3.767	3.317	7.333	8.900	4	5
Plethodon dunni	5	61.540	57.420	10.260	6.460	5.700	12.260	15.240	4	5
Plethodon elongatus	2	51.250	54.300	9.500	5.000	4.450	9.100	10.650	4	5
Plethodon glutinosus	10	73.510	78.420	12.860	7.680	7.710	16.200	18.910	4	5
Plethodon grobmani	9	60.200	57.467	11.111	6.567	6.211	13.056	16.156	4	5
Plethodon hoffmani	10	49.620	50.680	7.580	4.180	3.660	7.580	9.610	4	5
Plethodon hubrichti	10	53.830	59.700	8.200	4.810	4.160	9.380	11.600	4	5
Plethodon idahoensis	2	43.200	45.900	8.650	4.850	4.350	9.950	11.600	4	5
Plethodon jordani	9	68.500	67.900	11.522	7.344	7.033	15.056	18.822	4	5
Plethodon kentucki	6	63.317	67.683	11.700	6.967	5.950	14.900	17.900	4	5
Plethodon mississippi	10	67.520	65.700	11.590	6.850	6.680	13.600	16.530	4	5
Plethodon neomexicanus	10	62.050	56.570	9.960	5.240	4.790	9.860	11.230	4	5
Plethodon nettingi	10	45.050	44.260	7.410	4.330	3.420	8.360	10.330	4	5
Plethodon ouachitae	2	57.300	55.100	11.400	6.900	5.450	13.300	16.050	4	5
Plethodon punctatus	11	64.091	71.791	11.455	6.809	5.991	14.691	17.509	4	5
Plethodon richmondi	10	52.460	51.550	7.180	4.080	3.720	6.500	8.170	4	5
Plethodon serratus	3	40.433	29.333	6.700	3.867	3.333	6.733	7.933	4	5
Plethodon shenandoah	5	46.900	42.700	7.800	4.480	3.920	9.000	11.100	4	5
Plethodon teyahalee	4	72.100	60.800	11.900	6.300	6.300	14.800	19.400	4	5
Plethodon vandykei	3	53.700	43.867	8.700	5.967	5.033	12.833	15.167	4	5
Plethodon vehiculum	3	55.467	46.467	8.633	5.233	4.800	9.933	12.067	4	5
Plethodon ventralis	10	42.000	35.920	6.510	3.960	3.270	7.190	8.760	4	5
Plethodon wehrlei	10	57.470	55.910	9.680	5.840	4.980	12.190	14.690	4	5
Plethodon welleri	10	42.700	32.440	7.360	4.100	3.780	7.770	9.740	4	5
Plethodon yonahlossee	12	79.342	78.175	14.267	8.908	8.242	19.875	23.792	4	5
Pseudoeurycea bellii	3	73.633	51.967	14.333	9.600	8.400	16.567	17.267	4	5
Pseudoeurycea cephalica	3	44.833	35.800	9.033	5.900	4.633	11.300	13.000	4	5
Pseudoeurycea gadovii	3	27.600	20.467	5.933	4.200	3.067	7.000	7.867	4	5
Pseudoeurycea galeanae	1	50.600	32.400	9.800	6.400	5.700	12.400	14.300	4	5
Pseudoeurycea leprosa	4	58.125	47.300	8.650	5.575	5.400	11.200	11.675	4	5
Pseudoeurycea rex	1	32.800	18.900	7.400	3.800	3.100	7.200	7.900	4	5
Pseudoeurycea robertsi	1	47.700	24.300	9.600	5.500	5.300	11.400	12.700	4	5
Pseudoeurycea werleri	5	53.020	45.240	12.820	6.580	6.620	12.460	13.820	4	5
Pseudotriton montanus	10	74.950	52.350	11.660	6.850	7.840	12.590	15.870	4	5

Table B1. (Contd.)

Species	N	Snout-vent length	Tail length	Head length	Snout-eye length	Body width	Forelimb length	Hindlimb length	Fingers	Toes
Pseudotriton ruber	10	82.750	49.750	13.320	7.590	8.890	13.040	16.030	4	5
Stereochilus marginatus	1	57.700	49.600	8.200	5.400	3.900	9.200	11.100	4	5
Thorius macdougalli	5	19.260	18.300	3.760	2.340	1.880	3.560	4.060	4	5
Thorius pennatulus	5	20.800	31.140	4.260	2.200	1.920	3.220	3.740	4	5
Thorius pulmonaris	1	22.100	21.600	4.000	2.400	1.900	3.900	3.600	4	5
Proteidae										
Necturus alabamensis	4	131.925	58.600	27.125	11.175	13.475	16.625	19.225	4	4
Necturus beyeri	5	137.920	61.880	29.640	12.460	14.680	19.040	18.020	4	4
Necturus maculosus	7	186.171	80.843	30.886	17.343	22.486	28.457	29.714	4	4
Necturus punctatus	4	126.100	50.800	25.100	12.500	14.700	17.725	17.625	4	4
Proteus anguinus	1	146.800	49.700	14.100	8.600	6.400	17.800	15.200	3	2
Rhyacotritonidae										
Rhyacotriton cascadae	10	47.150	31.100	8.790	5.610	4.830	11.110	12.480	4	5
Rhyacotriton kezeri	2	45.400	26.600	7.850	5.850	4.900	8.750	11.550	4	5
Rhyacotriton olympicus	5	48.960	30.740	8.400	5.820	5.160	10.260	12.380	4	5
Rhyacotriton variegatus	3	44.600	27.167	8.500	5.833	5.333	10.000	12.067	4	5
Salamandridae										
Calotriton asper	3	57.867	51.433	13.633	7.667	7.667	16.333	15.833	4	5
Chioglossa lusitanica	2	45.850	73.400	8.000	4.750	5.000	10.200	11.150	4	5
Cynops ensicauda	5	60.820	67.120	13.740	8.680	9.020	20.560	20.560	4	5
Cynops pyrrhogaster	10	63.780	63.720	13.890	8.630	9.700	21.110	21.060	4	5
Echinotriton andersoni	2	82.500	79.600	18.450	11.950	17.300	24.850	26.150	4	5
Lissotriton boscai	4	30.500	30.000	7.875	4.750	3.350	11.300	11.700	4	5
Lissotriton helveticus	4	40.400	32.825	9.450	6.125	6.325	14.000	13.650	4	5
Lissotriton montandoni	2	44.300	36.350	9.800	6.500	5.350	15.900	15.250	4	5
Lissotriton vulgaris	9	40.644	37.756	9.156	5.900	5.389	13.922	13.900	4	5
Mertensiella caucasica	1	73.900	77.200	15.300	11.400	10.200	24.700	26.700	4	5
Mesotriton alpestris	8	47.063	35.412	10.463	6.625	7.650	15.837	15.562	4	5
Neurergus crocatus	3	75.233	71.067	15.433	10.433	10.400	25.000	28.300	4	5
Neurergus strauchii	2	74.300	74.650	15.750	10.100	12.500	24.150	26.300	4	5
Notophthalmus meridionalis	3	44.400	39.933	9.733	6.533	6.167	13.967	14.033	4	5
Notophthalmus perstriatus	2	43.250	48.300	10.750	7.100	4.950	13.700	15.000	4	5
Notophthalmus viridescens	10	54.180	62.070	12.470	7.270	8.390	17.430	17.960	4	5
Ommatotriton vittatus	1	49.000	37.800	10.200	6.700	5.000	16.900	20.700	4	5
Pachytriton brevipes	2	99.750	89.400	18.000	10.600	13.250	19.250	25.650	4	5
Paramesotriton caudopunctatus	1	68.600	62.900	18.700	11.100	9.500	20.800	21.400	4	5
Paramesotriton chinensis	2	64.250	60.100	16.700	10.050	8.850	22.800	23.650	4	5
Paramesotriton hongkongensis	2	78.450	70.050	20.250	12.850	12.850	23.200	23.600	4	5
Pleurodeles waltl	8	90.425	87.788	20.837	11.588	19.388	24.250	29.562	4	5
Salamandra atra	3	66.767	45.467	12.533	8.700	7.600	20.033	24.200	4	5
Salamandra salamandra bejarae	9	117.089	70.267	22.333	14.722	21.033	31.567	34.089	4	5
Salamandrina terdigitata	10	33.970	45.840	7.640	5.110	3.500	10.130	10.680	4	4
Taricha granulosa	8	73.250	88.325	15.012	9.638	10.188	22.488	23.438	4	5
Taricha rivularis	2	69.100	84.200	14.750	10.150	10.200	24.950	25.750	4	5
Taricha torosa	10	77.610	93.480	16.610	10.620	11.760	26.350	27.430	4	5
Triturus cristatus	8	63.625	43.863	11.650	7.200	6.575	17.438	19.337	4	5
Triturus pygmaeus	10	54.900	43.030	12.550	7.840	8.370	17.590	18.550	4	5
Tylototriton kweichowensis	5	87.120	75.200	20.000	10.840	15.780	25.160	27.700	4	5
Tylototriton verrucosus	4	69.350	61.250	16.300	10.300	11.675	21.975	26.175	4	5
Sirenidae										
Pseudobrancus axanthus	10	90.140	59.490	7.470	3.680	3.760	3.760	0.000	3	0
Siren intermedia	5	197.200	100.220	21.040	10.760	12.400	16.380	0.000	4	0
Siren lacertina	5	257.800	141.040	25.680	12.460	18.320	19.960	0.000	4	0