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RESEARCH PERSPECTIVES: STASIS SHMASIS - WHAT SALAMANDERS WERE REALLY DOING IN THE YULE LOG

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Whether it is their nocturnal habits, their ability to regenerate entire limbs and tails with functional neurons, or simply their ability to emerge unsinged from a burning yule log, salamanders have long intrigued humans and witches alike. Salamanders are marvelous beasts in many other ways. They have the widest range of feeding modes of any vertebrates (Wake and Deban 2000, Deban 2002), from gaping and sucking like most fish (Deban and Wake 2000), to insanely ballistic, sticky tongues (Deban et al. 1997). Salamander life histories include obligately paedomorphic forms that retain gills and remain aquatic throughout life even as adults, to forms that metamorphose from a gilled larva to an aquatic or terrestrial form with or without lungs (the most diverse salamander family – the Plethodontidae – lack lungs and respire completely through their skin), to species that have direct development, to livebearers (Tilley and Bernardo 1993; Sever 2003). The smallest adult salamanders hide in Mexican forests and are barely more than a centimeter in body length (Hanken et al. 2004), the largest are two meter long monsters marauding in large rivers of China and Japan (Okada 2001). Salamanders inhabit caves, aquifers, ponds, lakes, streams, rivers, are subterranean burrowers and epigean stalkers and arboreal acrobats. No other lineage of vertebrates, if not metazoans, rivals the diversity of salamander ecological strategies along so many different axes. Salamanders are also important model systems in many areas of behavioral, ecological and evolutionary research, and are one of the closest extant windows into the evolution of terrestriality in vertebrates.

Three recent papers (Mueller et al. 2004; Chippindale et al. 2004; Wiens et al. 2005) now reveal how little we understood about salamander evolution, and hold far-reaching lessons for evo-devo, the pace, reversibility and rampant convergence of morphological evolution, and the implications of both of these areas for phylogenetic inference.

Salamander morphological evolution is paradoxical. On one hand salamanders are viewed as paragons of morphological stasis (Gao and Shubin 2003). The earliest (and phylogenetically most basal) salamander fossils are congeneric with and morphologically virtually identical to extant North American hellbenders (Gao and Shubin 2003; see also Evans et al. 2005). Moreover, salamanders are renowned for harboring levels of genetic variation that typically characterize generic or family level divergence in other vertebrates (Highton 1995, 2000; García-París et al. 2000; Parra-Olea and Wake 2001; Tilley and Mahoney 1996; Parra-Olea et al. 2004) and the pace of discovery of cryptic salamander diversity is undampened (Frost 2004).

The new papers reveal the other side of this paradox at two levels – within the speciose Plethodontidae (Mueller et al. 2004; Chippindale et al., 2004), and among the salamander families (Wiens et al. 2005) – and show that salamander evolution has been more of a see-saw of repeated evolution, loss, and re-evolution of particular morphologies suited to different ecological milieus, but within the conserved salamander Bauplan. Of four presumed major lineages within plethodontids (Dujnn 1926; Wake 1966; Frost 2004; Mueller et al. 2004; Chippindale et al., 2004), the subfamily Desmognathinae contains mostly species having aquatic larvae, but a few having direct development (Tilley and Bernardo 1993). The remaining lineages are tribes within subfamily Plethodontinae: Hemidactyliini contains only species having an aquatic larval stage and both metamorphosing and paedomorphic species; Plethodontini and the Bolitoglossini contain only directly-developing species (Figure 1A). Diversification among these lineages was long cast in a classically progressive light, reminiscent of the vertebrate transition to land (Dunn 1926; Wake 1966). Thus Desmognathines, in which most species have an aquatic larval stage, were thought basal to Plethodontines, and Hemidactyliini, also having complex life cycles and paedomorphic species, was placed basally within Plethodontinae. Directly-developing species were considered more derived, culminating in the spectacular radiation of Bolitoglossines (‘missile tongues’) in tropical America, in part driven by developmental repatternings that permitted evolution of remarkable terrestrial adaptations (Wake 1966; Deban et al. 1997; Parra-Olea and Wake 2001). Mueller et al. (2004) evaluated this scenario using whole mitochondrial genomes of 22 plethodontid taxa, whereas Chippindale et al. (2004) used a larger character matrix comprising 123

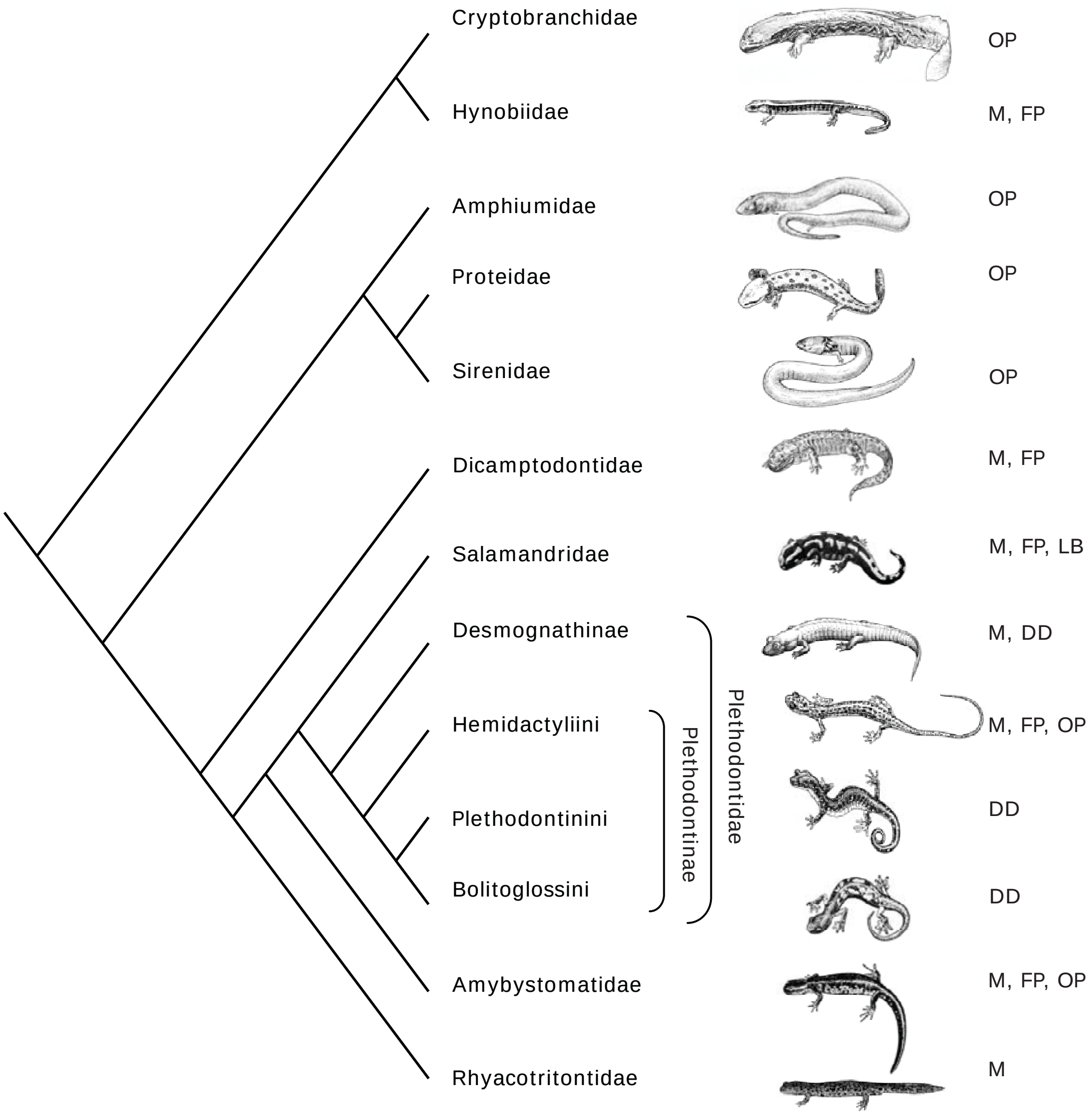

Figure 1A. Traditional hypothesis of relationships of salamander families, and lineages within Plethodontidae based upon morphological features (after Wake 1966; Gao and Shubin 2003) and summarizing life history states exhibited within each lineage (CLC = complex life cycle with a metamorphosis from a larva to a terrestrial or aquatic adult morphology; OP = obligately paedomorphic; FP = facultatively paedomorphic; DD = direct development, development external to parent; LB = livebearing). This hypothesis shows the obligately paedomorphic families to be basal relative to most other families, and three of the four families forming a distinct, fairly basal clade. Note that salamander illustrations are not to the same scale.

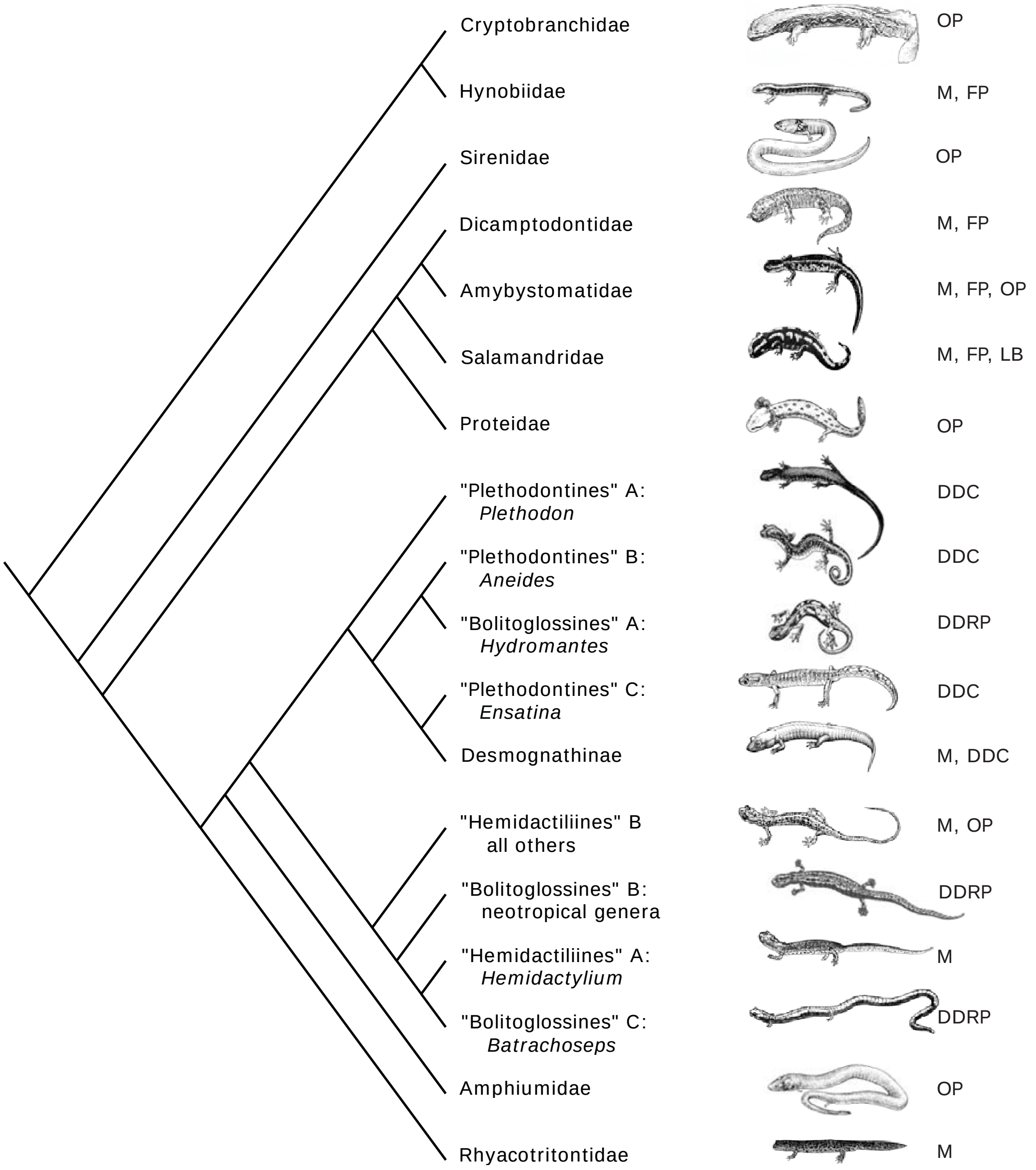

Figure 1B. Synthesis of new findings about salamander family level relationships and phylogenetic diversity within Plethodontidae based on an enlarged data set comprising many more morphological characters and both mitochondrial and nuclear gene sequences (after Figure 2 in Mueller et al. 2004; Figure 2 in Chippindale et al. 2004; Figure 8 in Wiens et al. 2005). Expansion of the Plethodontinae reveals greater complexity in the evolution of life histories, including multiple origins of direct development and re-evolution of an aquatic larva and complex life cycle from within a clade of direct developers. Abbreviations as in Figure 1A except DDC = direct development via condensation of the larval stage, DDRP = direct development involving significant embryonic repatterning. Note that salamander illustrations are not to the same scale.

morphological characters, 1,473 bp of mitochondrial sequences, and 1,525 bp of a conservatively evolving nuclear gene scored across 31 ingroup taxa.

Their findings were largely parallel and astonishing at several levels (Figure 1.B). First, both studies found that Desmognathines nest within Plethodontini, rendering the latter paraphyletic and indicating that a complex life cycle with its anatomically specialized larval form has re-evolved from a directly-developing ancestor (Figure 1B). Second, both studies found that the sister taxon of the highly derived “bolitoglossines” is a “hemidactyliine” species (Hemidactylium) with a complex life cycle, not the directly-developing “plethodontines”. Thus Hemidactyliini is paraphyletic as well. Finally, *Hydromantes*, the “bolitoglossine” with the ballistic tongue quite clearly nests within a clade containing some directly-developing “plethodontines” + desmognathines – and is not part of the major evolutionary radiation of central American “bolitoglossines”; Thus “Bolitoglossini”, too, is paraphyletic, and their characteristic elaborate tongue protrusion mechanisms, and the developmental repatterning the permits them, must have evolved at least twice!

Hence, the complex life cycle and thus, the aquatic larval morphology must have evolved twice within Plethodontines– once basally but again from within a lineage of directly developing forms that had foregone the archetypal amphibian reliance on water. Although reversals of all sorts of phenotypes are common (e.g., eye loss, leg loss), re-evolution of complex functional complexes such as a larval morphology appears to be rare (Porter and Crandall 2003). Just as snakes were thought to have never re-evolved legs (Tchernov et al. 2000), it seemed impossible that a complex life cycle could re-evolve from a lineage that had mastered terrestriality to the extent that it had forsaken the aquatic environment. But re-evolution of complex traits now seems much more a function of the ways in which developmental shifts arise and are regulated at the molecular level, rather than a function of the complexity of a morphological feature per sé. As Chippindale et al. (2004) suggest, this reversal probably relates not only to the availability of an aquatic adaptive zone, but to the way in which direct development was accomplished in Desmognathine ancestors, via condensation of the larval stage (Figure 1).

The third paper (Wiens et al. 2005) tackles the unresolved issue of relationships among the salamander families using the largest morphological character matrix ever assembled (326 characters), as well as new nuclear gene sequences and additional taxon sampling compared to previous analyses. A longstanding challenge in reconstructing salamander familial relationships has been the prevalence of paedomorphosis among several families which makes scoring of homologous adult morphological features impossible. This has resulted in reliance upon paedomorphic characters to the exclusion of most others, so that these characters dominate the evolutionary signal in most analyses, and strongly support a close relationship among paedomorphic families (Figure 1A.). This problem was overcome by generating several hundred new morphological characters, permitting explicit tests for effects of paedomorphic characters. Paedomorphic traits were found to cause an erroneous phylogenetic grouping which included not only the paedomorphic families, but the individual paedomorphic species from other families in which most species metamorphose. Moreover, this conclusion was strongly statistically supported when the character matrix had a high proportion of paedomorphic characters. In contrast, the substantially enlarged data matrix exposed the homoplasious signal of the paedomorphic traits, and thus also showed that these families have evolved paedomorphic features convergently.

Two clear lessons for phylogenetic inference emerged from this study; One is that convergent reversals can be not only misleading, but statistically well-supported. The second it that this finding did not require inclusion of molecular characters; It is a testimony to the power of sampling, not a battle between morphology versus molecular data in phylogenetic inference – although the molecular characters, it can be argued, seem to be fundamentally less susceptible to the homplasious effects of similar selective regimes; they at least seem less strongly correlated with morphological evolution.

Other general lessons emerge from these studies ranging from the evolution and expression of developmental programs, to the prevalence of convergence, to the dangers of typological thinking, and the implications of all of these for phylogenetic analysis.

One implication is that despite the genomic conservatism in developmental processes across animals, spatiotemporal patterns of gene expression and regulation are tremendously variable and do not form an ordered sequence – so their phenotypic manifestations may often be misleading in phylogenetic inference. Evo-devo analyses thus must guard against equating ordered ontogenetic sequences with phylogenetic patterns, for such ordered typology may often mislead. These convergent patterns of paedomorphic morphologies may nonetheless be seized as opportunities for examining whether mechanisms of gene expression and regulation are also convergent, or whether different regulatory pathways yield these grossly similar phenotypes.

The ways these new studies must change long held views about salamander evolution hold interesting lessons for students of amphibians, but pertain to typological thinking in all areas of biology. One transformed view is that salamander life historical and morphological radiation has been more active and nonlinear than ever imagined. Life historical features are often viewed as evolving in a progressive sequence, but convergent life history adaptations are common (Tilley and Bernardo 1993). These studies demonstrate that convergence in morphological design for an aquatic habit is evident at many hierarchical levels within salamanders: Obligately paedomorphic forms, and the complex life cycle itself have repeatedly evolved, so do not simply represent the retention of symplesiomorphic features, nor does direct development necessarily represent the zenith of life historical specialization (Figure 1). Rather, the repeated evolution of a life historical feature in response to a particular selective milieu reflects the power of selection to modify organismal design via adaptive evolution – in the case of salamanders, the adaptive significance of the larval body plan - in either the context of a complex life cycle as in Desmognathines (Tilley and Bernardo 1993), or as paedomorphic adults among several families (Figure 1B) - as a specialized solution to aquatic life.

Salamander family ties have been confused for centuries in part because of typological thinking and a progressive bias in thinking about evolutionary transitions. A fundamental concern in phylogenetic analysis is extracting a signal of relationships from the noise arising from symplesiomorphies and homoplasy. A clear lesson from two of these papers (Chippindale et al. 2004; Wiens et al. 2005) is the import of large character matrices as a strategy detecting homoplasious signal. Only the cumulative signal that emerges from a large number of characters can reduce the distracting homoplasious noise that arises from rampant convergence and thus, permit its detection.

Another new insight is that, within the most diverse family, Plethodontidae, there is tremendous unappreciated deep diversity. Instead of four major lineages, there are nine distinct lineages among which there have been convergent changes in developmental programs and attendant convergent adaptations in tongue protrusion, life cycle structure and even brain architecture. But reversals may instigate further adaptive radiations. Morphological features determine how an organism makes its living, and it is the very close correspondence between morphology and functionality that leads us astray. Again here we see the efficacy of selection for adaptations to the aquatic adaptive zone, with re-evolution of a complex life cycle in the Desmognathinae permitting them to invade and then radiate within an unexploited adaptive zone in Appalachia (Tilley and Bernardo 1993).

Salamander morphological evolution is not paradoxical, but a testament to the spatio-temporally dynamic nature of selection and the adaptive potential of organisms to respond to it. Rather than thinking of salamanders as paragons of morphological stasis, these new studies compel us to see them as Darwinian adaptive monsters that have constantly refined their morphological groundplans to suit ever changing and newly available adaptive zones. Who knew that the Yule log was such a crucible of evolution?

LITERATURE CITED

Chippindale, P. T., R. M. Bonett A. S. Baldwin, and J. J. Wiens. 2004. Phylogenetic evidence for a major reversal in life history evolution in plethodontid salamanders. Evolution 58:2809-2822.

Deban, S. M. and D. B. Wake. 2000. Aquatic Feeding in Salamanders, In: Feeding: form, function and evolution in tetrapod vertebrates. K. Schwenk, ed., Academic Press, San Diego. 65-94.

Deban, S. M. 2002. Constraint and convergence in the evolution of salamander feeding. In: Vertebrate Biomechanics and Evolution, J.-P. Gasc, A. Casinos, and V.L. Bels, eds., BIOS Scientific Publishers, Oxford. 163-180.

Deban, S. M., D. B. Wake, AND G. Roth. 1997. Salamander with a ballistic tongue. Nature 38:27-28.

Dunn, E. R. 1926. Salamanders of the family Plethodontidae. Smith College Fiftieth Anniversary Publications. Northampton, Massachusetts. 446 pp.

Evans, S. E., C. Lally, D. C. Chure, A. Elder, and J. A. Maisano. 2005. A late Jurassic salamander (Amphibia: Caudata) from the Morrison Formation of North America. Zoological Journal of the Linnean Society 143:599-616.

Frost, Darrel R. 2004. Amphibian Species of the World: an Online Reference. Version 3.0 (22 August, 2004). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

Gao, K. and Shubin, N. S. 2003. Earliest known crown group salamanders. Nature 422:424-429.

Garcia-Paris, M., D. A. Good, G. Parra-Olea, and D. B. Wake. 2000. Biodiversity of Costa Rican salamanders: Implications of high levels of genetic differentiation and phylogeographic structure for species formation. Proc. Natl. Acad. Sci. USA 97:1640-1647.

Hanken, J., D. B. Wake, and H. L. Freeman. 1999. Three new species of minute salamanders (*Thorius*: Plethodontidae) from Guerrero, México, including the report of a novel dental polymorphism in urodeles. Copeia 1999:917-931.

Highton, R. 1995. Speciation in eastern North American salamanders of the genus *Plethodon*. Annual Review of Ecology and Systematics, 26: 579-600.

Highton, R. 2000. Detecting cryptic species using allozyme data. Pp. 215-241. In R. C. Bruce, R. G. Jaeger, and L. D. Houck (Eds.). The Biology of Plethodontid Salamanders. Kluwer Academic/Plenum Publishers. New York, NY.

Mueller, R. L., J. R. Macey, M. Jaekel, D. B. Wake, and J. L. Boore. 2004. Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. Proceedings of the National Academy of Science USA, 101:13820-13825.

Okada, S. 2004. The *Andrias* Homepage. Copyright Sumio Okada; available on the world wide web at: http://www3.ocn.ne.jp/~herpsgh/aboutandrias.html

Parra-Olea, G., and D. B. Wake. 2001. Extreme morphological and ecological homoplasy in tropical salamanders. Proceedings of the National Academy of Science USA 98:7888-7891.

Parra-Olea, G., M. Garcia-Paris, and D. B. Wake. 2004. Molecular diversification of salamanders of the tropical American genus *Bolitoglossa* (Caudata: Plethodontidae) and its evolutionary and biogeographical implications. Biological Journal of the Linnaean Society 81:325-346.

Porter, M. L. and K. A. Crandall. 2003. Lost along the way: The significance of evolution in reverse. TREE 18:541-547.

Sever, D. M. (ed.) 2003. Reproductive Biology and Phylogeny of Urodela (Amphibia). Science Publishers, Inc. Enfield, New Hampshire.

Tchernov, E., O. Rieppel, H. Zaher, M. J. Polcyn, and L. L. Jacobs. 2000. A fossil sanke with limbs. Science 287:2010-2012.

Tilley, S. G. and J. Bernardo. 1993. Life history evolution in plethodontid salamanders. Herpetologica 49:154-163.

Tilley, S. G. and M. J. Mahoney. 1996. Patterns of genetic differentiation in salamanders of the *Desmognathus ochrophaeus* complex (Amphibia: Plethodontidae). Herp. Monogr. 10:1-42.

Wake, D. B. 1966. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Memoirs of the Southern California Academy of Sciences 4:1-111.

Wake, D. B. and S. M. Deban. 2000. Terrestrial feeding in salamanders, In: Feeding: Form, function and evolution in tetrapod vertebrates. K. Schwenk, ed., Academic Press, San Diego. 95-116.

Wiens, J. J., R. M. Bonett, and P. T. Chippindale. 2005. Ontogeny discombobulates phylogeny: Paedomorphosis and higher-level salamander phylogeny. Systematic Biology 54(1):91-110.