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Molecular Phylogenetics and Evolution 41 (2006) 368–383

MOLECULAR PHYLOGENETICS AND EVOLUTION

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A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata)

David W. Weisrock [a,*], Theodore J. Papenfuss [b], J. Robert Macey [b], Spartak N. Litvinchuk [c], Rosa Polymeni [d], Ismail H. Ugurtas [e], Ermi Zhao [f], Houman Jowkar [g], Allan Larson [a]

[a] *Department of Biology, Box 1137, Washington University, Saint Louis, MO 63130, USA*
[b] *Museum of Vertebrate Zoology, 3101 Valley Life Science Building, University of California, Berkeley, CA 94720, USA*
[c] *Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Pr., 4, 194064 St. Petersburg, Russia*
[d] *Department of Zoology and Marine Biology, Faculty of Biology, School of Science, University of Athens, GR-15784 Panepistimioupolis, Athens, Greece*
[e] *Department of Biology, Uludag University, 16059 Bursa, Turkey*
[f] *Chengdu Institute of Biology, Academia Sinica, Chengdu, Sichuan, China*
[g] *Tehran University, Tehran, Iran*

Received 13 January 2006; revised 4 May 2006; accepted 9 May 2006

Abstract

We examine phylogenetic relationships among salamanders of the family Salamandridae using approximately 2700 bases of new mtDNA sequence data (the tRNA^{Leu}, ND1, tRNA^{Ile}, tRNA^{Gln}, tRNA^{Met}, ND2, tRNA^{Trp}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, and COI genes and the origin for light-strand replication) collected from 96 individuals representing 61 of the 66 recognized salamandrid species and outgroups. Phylogenetic analyses using maximum parsimony and Bayesian analysis are performed on the new data alone and combined with previously reported sequences from other parts of the mitochondrial genome. The basal phylogenetic split is a polytomy of lineages ancestral to (1) the Italian newt *Salamandrina terdigitata*, (2) a strongly supported clade comprising the "true" salamanders (genera *Chioglossa*, *Mertensiella*, *Lyciasalamandra*, and *Salamandra*), and (3) a strongly supported clade comprising all newts except *S. terdigitata*. Strongly supported clades within the true salamanders include monophyly of each genus and grouping *Chioglossa* and *Mertensiella* as the sister taxon to a clade comprising *Lyciasalamandra* and *Salamandra*. Among newts, genera *Echinotriton*, *Pleurodeles*, and *Tylototriton* form a strongly supported clade whose sister taxon comprises the genera *Calotriton*, *Cynops*, *Euproctus*, *Neurergus*, *Notophthalmus*, *Pachytriton*, *Paramesotriton*, *Taricha*, and *Triturus*. Our results strongly support monophyly of all polytypic newt genera except *Paramesotriton* and *Triturus*, which appear paraphyletic, and *Calotriton*, for which only one of the two species is sampled. Other well-supported clades within newts include (1) Asian genera *Cynops*, *Pachytriton*, and *Paramesotriton*, (2) North American genera *Notophthalmus* and *Taricha*, (3) the *Triturus vulgaris* species group, and (4) the *Triturus cristatus* species group; some additional groupings appear strong in Bayesian but not parsimony analyses. Rates of lineage accumulation through time are evaluated using this nearly comprehensive sampling of salamandrid species-level lineages. Rate of lineage accumulation appears constant throughout salamandrid evolutionary history with no obvious fluctuations associated with origins of morphological or ecological novelties.

Keywords: Lineage accumulation; Mitochondrial DNA; Newt; Salamander; Salamandridae

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[*] Corresponding author. Present address: Department of Biology, University of Kentucky, 101 Morgan Building, Lexington, KY, 40506-0225, USA. Fax: +859 257 1717.
E-mail address: weisrock@uky.edu (D.W. Weisrock).

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doi:10.1016/j.ympev.2006.05.008

1. Introduction

The salamander family Salamandridae, comprising 16 genera and 66 recognized species, represents one of the most diverse groups of extant salamanders. Salamandrids have the largest geographic distribution of any salamander family, extending across the holarctic continents of Asia, Europe, and North America with a small and recent expansion into North Africa. The Salamandridae, which contains the traditionally recognized newts (salamanders with rough keratinized skin) and the "true" salamanders (smooth-skinned salamandrids), has diversified in both terrestrial and aquatic environments through a variety of derived feeding morphologies (Özeti and Wake, 1969; Wake and Özeti, 1969), and courtship behaviors (Salthe, 1967). The historical association between these evolutionary derivations and rates of lineage accumulation (Schluter, 2000) remains to be measured. The salamandrid fossil record is sparse, requiring that rates of lineage accumulation be estimated from systematic studies of extant populations.

Molecular phylogenies are an important framework for studying the tempo of lineage diversification (Slowinski and Guyer, 1989; Mooers and Heard, 1997; Nee et al., 1994; Sanderson and Donoghue, 1996). Plotting lineage accumulation as a function of estimated divergence time and integrating this information with null models of the birth and death of lineages (Nee et al., 1992) permit statistical testing of hypotheses of lineage diversification over time (Paradis, 1997; Pybus and Harvey, 2000; Pybus et al., 2002). These phylogenetic approaches have yielded important insight in the tempo of evolutionary diversification in diverse organismal groups including iguanian lizards (Harmon et al., 2003), marine fishes (Ruber and Zardoya, 2005), mosses (Shaw et al., 2003), and plethodontid salamanders (Kozak et al., 2006).

No single phylogenetic study has sampled all salamandrid species. The most complete prior study (Titus and Larson, 1995) used a combination of morphological and mitochondrial DNA (mtDNA) (12S and 16S rDNA and the intervening tRNA^{Val} gene) characters from 18 species. This study provided strong support for monophyly of the Salamandridae and for some intergeneric groupings, which were congruent with molecular phylogenetic results for 10 genera reported by Frost et al. (2006). Monophyly was statistically rejected for the genera *Mertensiella* and *Triturus*. However, there was little support for many basal relationships within the family, particularly for the placement of the monotypic newt genus *Salamandrina*.

Phylogenetic relationships within many salamandrid groups have received considerable attention (e.g. Caccone et al., 1997; Carranza and Amat, 2005; Chan et al., 2001; Lu et al., 2004; Steinfartz et al., 2000, 2002; Veith et al., 2004; Weisrock et al., 2001), yet many species-level relationships require further resolution. Evolution of the genus *Triturus* has been studied extensively (Halliday and Arano, 1991), yet phylogenetic resolution among species remains ambiguous, even with a host of morphological, molecular, and behavioral data (Giacomo and Balletto, 1988; Macgregor et al., 1990; Rafinski and Arntzen, 1987; Zajc and Arntzen, 1999). Monophyly of the genus *Triturus* was rejected by the mtDNA studies of Titus and Larson (1995), based on two species. However, studies using more comprehensive ingroup sampling, but limited outgroup sampling have found *Triturus* to be either monophyletic or paraphyletic (e.g. Zajc and Arntzen, 1999). Recent studies of the genus *Euproctus* indicate that it is not monophyletic (Caccone et al., 1994, 1997; Carranza and Amat, 2005), and instead may represent two phylogenetically divergent groups, one of which was recently placed in the genus *Calotriton* (Carranza and Amat, 2005). A thorough phylogenetic assessment of these genera and other salamandrid lineages requires comprehensive species-level sampling of the entire family.

We present a nearly comprehensive species-level sampling of the Salamandridae in conjunction with new and previously published mtDNA sequence data to address both the deep phylogenetic relationships among major lineages of salamandrids and the relationships among the more recently derived lineages. The resulting phylogenies are then used to measure the tempo of lineage diversification across the history of the Salamandridae.

2. Materials and methods

2.1. Taxon sampling and data collection

This study used approximately 2700 bases of new mtDNA sequence data collected from 96 individuals including 61 of the 66 recognized salamandrid species and outgroups. Five salamandrid species were not included: *Triturus helveticus*, *Triturus italicus*, *Calotriton arnoldi*, *Cynops chenggongensis*, and *Cynops wolterstorfii*. The latter species is considered to be recently extinct (Zhao, 1998). We follow the taxonomic suggestion of Veith and Steinfartz (2004) in placing *Mertensiella luschani* and related species formerly considered subspecies of *M. luschani* in a new genus, *Lyciasalamandra*, based on mtDNA-based statistical support for the nonmonophyly of the previously recognized genus *Mertensiella* (Weisrock et al., 2001) and corroborating allozyme-based genetic evidence (Veith and Steinfartz, 2004).

Sequence data were collected from a contiguous block of genes including the tRNA^{Leu}, ND1, tRNA^{Ile}, tRNA^{Gln}, tRNA^{Met}, ND2, tRNA^{Trp}, tRNA^{Ala}, tRNA^{Asn} genes, the origin for light-strand replication (O$_L$), and the tRNA^{Cys}, tRNA^{Tyr}, and COI genes (hereafter called the tRNA^{Leu}–COI genic region). All genes included are full-length except for COI, which contained approximately 30 bases of 5′ partial sequence. This gene region is similar to the one used in an earlier study of the "true" salamanders (Weisrock et al., 2001), except that it contains approximately 670 additional bases of sequence completing the 5′ portion of the ND1 gene and the preceding tRNA^{Leu} gene. These additional sequences were generated for individuals used by Weisrock et al., 2001 and added to their GenBank records. DNA