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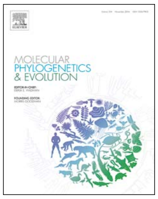

Paleoclimate determines diversification patterns in the fossorial snake family Uropeltidae Cuvier, 1829

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ABSTRACT

Understanding how and why diversification rates vary across evolutionary time is central to understanding how biodiversity is generated and maintained. Recent mathematical models that allow estimation of diversification rates across time from reconstructed phylogenies have enabled us to make inferences on how biodiversity copes with environmental change. Here, we explore patterns of temporal diversification in Uropeltidae, a diverse fossorial snake family. We generate a time-calibrated phylogenetic hypothesis for Uropeltidae and show a significant correlation between diversification rate and paleotemperature during the Cenozoic. We show that the temporal diversification pattern of this group is punctuated by one rate shift event with a decrease in diversification and turnover rate between ca. 11 Ma to present, but there is no strong support for mass extinction events. The analysis indicates higher turnover during periods of drastic climatic fluctuations and reduced diversification rates associated with contraction and fragmentation of forest habitats during the late Miocene. Our study highlights the influence of environmental fluctuations on diversification rates in fossorial taxa such as uropeltids, and raises conservation concerns related to present rate of climate change.

1. Introduction

Environmental factors play a key role in mediating diversification processes by driving microevolutionary change and shaping phenotypic evolution. Until recently, researchers had to rely on the paleontological record to understand how biotic and abiotic factors have influenced spatio-temporal diversification patterns (Barnosky, 2001; Benton, 2009; Vermeij, 1994, 1987). Fossil data are, however, incomplete, unevenly distributed in space and time and are not available for many taxa, limiting inferences on diversification processes to certain taxa (Benton et al., 2000; Valentine et al., 2006). Recently, the advent of mathematical models and analytical tools to estimate speciation and extinction rates from reconstructed phylogenies (Etienne et al., 2012; Morlon et al., 2011; Nee et al., 1994b; Rabosky, 2014, 2006; Stadler, 2011) have revolutionized the study of historical diversification patterns. These methods have allowed more precise inferences on the underlying evolutionary mechanisms and the factors that influence diversification rates.

Although fossil evidence suggests that the environment has had a strong influence on speciation and extinction dynamics (Ezard et al., 2011), the potential role of paleoclimatic fluctuations in influencing diversification rates in different taxa has not been explored in great detail. Climatic fluctuations can trigger shifts in species ranges (Barry et al., 1995; Thomas and Lennon, 1999) and thereby influence lineage diversification (Hou et al., 2011; Kolář et al., 2016; Pepper et al., 2011). Climate is also a driver of mass extinctions (Ivany et al., 2000; Lewis et al., 2008; Thomas, 1990), which not only prune branches off the tree of life (Green et al., 2011), but also influence lineages that survive by increasing ecological opportunity and thus enhancing diversification (Krug et al., 2009). A few empirical studies employing recently developed analytical tools to estimate diversification rates from molecular phylogenies suggest that paleoclimatic fluctuations have had a pronounced effect on diversification rates in several taxa (e.g. Birds – Claramunt and Cracraft, 2015; Jetz et al., 2012; Insects – Condamine et al., 2016; Spiny-rayed fish – Near et al., 2012; Amphibians – Roelants et al., 2007; Mammals – Stadler, 2011). Other studies have highlighted the pivotal role of mass extinctions in diversification rate variation (Krug et al., 2009; Longrich et al., 2012). Such studies, however, have been predominantly on aquatic and above ground terrestrial taxa. Fossorial taxa (i.e. those that are predominantly subterranean and burrow into the ground) have been largely ignored. The subterranean environment is structurally simple and thought to be relatively stable compared to above ground in terms of environmental fluctuations, and thus thought to persist relatively unchanged for millions of years

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(Gibert and Deharveng, 2002). However, how environmental factors influence temporal diversification in fossorial taxa and the macroevolutionary implications of fossoriality remain unexplored.

We here investigate temporal diversification patterns of the snake family Uropeltidae, a lineage of highly specialized fossorial snakes found predominantly in the moist forests in peninsular India and Sri Lanka. Phylogenetically, Uropeltidae is within the major clade Alethinophidia. Morphology and molecular based phylogenies of snakes have consistently placed Uropeltidae sister to Cylindrophidae (Hsiang et al., 2015; Pyron et al., 2013a; Slowinski and Lawson, 2002; Streicher and Wiens, 2016; Vidal and Hedges, 2002; Wiens et al., 2008), although a few studies place Anomochilidae as the sister group to Uropeltidae (Hsiang et al., 2015; Lee et al., 2007; Lee and Scanlon, 2002 (Hsiang et al., 2015; Lee et al., 2007; Lee and Scanlon, 2002). Together, the clade comprising Uropeltidae, Cylindrophidae and Anomochilidae is generally referred to as Asian anilioids.

Presently the family Uropeltidae is represented by ca. 55 species within nine genera – *Melanophidium*, *Platyplectrurus*, *Teretrurus*, *Brachyophidium*, *Plectrurus*, *Pseudoplectrurus*, *Rhinophis*, *Pseudotyphlops* and *Uropeltis* (Wallach et al., 2014) with some authors considering *Pseudoplectrurus* as a synonym of *Rhinophis* (Pyron et al., 2016). The relationships among these genera have remained largely unknown, and morphology and molecular based phylogenetic analyses have resulted in unresolved polytomies (Bossuyt et al., 2004; Olori and Bell, 2012; Rieppel and Zaher, 2002). Here we present a robust genus level phylogenetic hypothesis along with divergence time estimates. We show that the temporal diversification pattern was influenced by a single diversification rate shift correlated with paleoclimatic events during the Cenozoic.

2. Materials and methods

2.1. Sampling

Sampling was carried out at multiple sites within the state of Kerala, Southern India. Collected samples were photographed, euthanized, fixed and preserved in ethanol, following which heart or liver tissues were collected and stored for DNA extraction. Additional tissue samples were obtained from ethanol preserved specimens deposited at the Zoological Survey of India – Western Ghat Regional Center (ZSI-WGRC).

2.2. DNA sequencing

Total genomic DNA was extracted using the standard Phenol-chloroform protocol (Bilton and Jaarola, 1996). One nuclear (c-mos) and three mitochondrial genes (12s rRNA, 16s rRNA and ND4) were amplified with the standard 3-step PCR protocol (Palumbi, 1996). We used hybrid primers with universal primer tails – T7 promoter (F) and T3 (R) attached to forward and reverse primers for PCR amplification (Appendix, Table A.1). PCR products were checked on an agarose gel, purified with ExoFastAP (Thermo Scientific™) and Sanger sequenced by Macrogen Inc, South Korea.

2.3. Phylogenetic analyses

A total of 71 taxa representing ~45 Operational Taxonomic Units (OTU's) (Uropeltidae + outgroups) were used for the analyses. Sequences of some uropeltids and their outgroups were obtained from Genbank (Appendix, Table A.3). Sequences of all the taxa for each gene were aligned separately using the MUSCLE algorithm (Edgar, 2004) implemented in MEGA6 (Tamura et al., 2013). Gaps were removed using Gap Strip/squeeze v2.1.0 (http://www.hiv.lanl.gov) with a gap tolerance of 75%, which deletes only columns that contain 75% gaps. Each gene dataset was checked for substitution saturation using DAMBE5 (Xia, 2013). The final multi-gene dataset containing 2121bp was concatenated using SequenceMatrix v1.8 (Vaidya et al., 2011). The dataset was partitioned by codon position and PartitionFinder v1.1.1 (Lanfear et al., 2012) was used to find the best fit models of nucleotide substitution (Appendix, Table A.4). A Maximum Likelihood analysis was carried out in RAxML GUI v1.3 (Silvestro and Michalak, 2012) with 1000 bootstrap replicates using default parameters. Bootstrap support was summarized as a 50% majority rule tree using Summtrees v4.0.0 (Sukumaran and Holder, 2015) implemented in the python library DendroPy v4.0.3 (Sukumaran and Holder, 2010). Clades with bootstrap values > 90 were considered as strongly supported, while those with values between 70 and 90 were considered as moderately supported. Bayesian inference of phylogeny was carried out in Mr. Bayes v3.2.0 (Ronquist et al., 2012) for the combined dataset with default priors using Markov chain Monte Carlo (MCMC) with two independent runs performed for 12.97 million generations, when the standard deviation of the split frequency fell below 0.01. MCMC chains were checked for convergence using Tracer v1.6 (Rambaut et al., 2014) and summarized as a 50% majority rule tree after discarding the first 25% of the samples as burn-in. Clades with posterior probabilities greater than 0.95 were considered as strongly supported.

2.4. Divergence dating

For the divergence dating analyses we obtained representative sequences for the four gene regions of all major lineages within Alethinophidia from Genbank to derive a dataset of 2133 bp with 116 taxa. We included additional taxa that were not part of the analyses in Section 2.3. These additions were needed because there was no reliable calibration source within the smaller dataset. We did not use this larger dataset for the RAxML and MrBayes analyses, because it has been shown that outgroup choice may affect tree topology within the ingroup (Kodandaramaiah et al., 2010; Wheeler, 1990).

Divergence times were estimated through Bayesian Inference implemented in BEAST v2.3.0 (Bouckaert et al., 2014). The combined dataset was partitioned into three bins as determined by TIGER v1.02 (Cummins and McInerney, 2011) and the best fit models were determined by PartitionFinder v1.1.1 (Appendix, Table A.4). We constrained the monophyly of three major clades and calibrated the tree by temporally constraining four nodes based on available fossil data (Appendix, Table A.5). Two independent analyses were run for 100 million generations sampling every 1000 generations. A relaxed uncorrelated lognormal clock model was used with a birth-death speciation prior. Other priors were set to their default values. The two runs were checked for convergence in Tracer v1.6 and the first 25% of each run was discarded as burn-in. We combined the two runs using LogCombiner v2.3.0 and constructed a maximum clade credibility tree using TreeAnnotator v2.3.0 in the BEAST2 package.

2.5. Diversification analyses

We pruned taxa from the ultrametric tree obtained from the divergence dating analyses to represent only the Asian anilioids, which included two species of Cylindrophidae, one species of Anomochilidae and 42 Operational Taxonomic Units within Uropeltidae (Appendix, Table A.6). We conducted all diversification analyses on three separate increasingly less inclusive trees pruned to represent the stem Asian anilioids, crown Asian anilioids and stem Uropeltidae to investigate the robustness of the temporal diversification pattern and rate estimates. The pruned datasets included 63% of the Asian anilioids which are presently represented by ca. 70 species and 75% of Uropeltidae which is represented by ca. 55 species.

We first plotted a Lineage through time (LTT) plot for the three trees. A visual inspection of the LTT plot of the stem Asian anilioids indicated an initial plateau ending at around 55 Ma and a smaller plateau around 34 Ma. Such plateaus in the LTT plots are often suggestive of relatively high background extinction or mass extinction