Molecular Phylogenetics and Evolution xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular data reveal spatial and temporal patterns of diversification and a cryptic new species of lowland *Stenocercus* Duméril & Bibron, 1837 (Squamata: Tropiduridae) ☆

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ARTICLE INFO

Article history:
Received 11 April 2015
Revised 28 July 2015
Accepted 9 September 2015
Available online xxxx

Keywords:
Andes
Climatic niche
Historical biogeography
Iguania
Stenocercini
Taxonomy

ABSTRACT

Phylogenetic studies have uncovered biogeographic patterns and the associated diversification processes of Neotropical wet forest taxa, yet the extensive open and drier biomes have received much less attention. In the *Stenocercus* lizard radiation, restricted sampling and phylogenetic information to date have limited inferences about the timing, spatial context, and environmental drivers of diversification in the open and dry lowland settings of eastern and southern South America. Based on new DNA sequence data of previously unsampled species, we provide an updated historical biogeographic hypothesis of *Stenocercus*. We infer phylogenetic relationships, estimate divergence times, and track ancestral distributions, asking whether cladogenetic events within the genus correlate to reported shifts in South American landscapes during the past 30 million years, focusing in the open and drier areas. To examine correlations between genetic and ecological divergence, we extracted environmental data from occurrence records and estimated climatic envelopes occupied by lowland taxa. Our results suggest that *Stenocercus* began to diversify around the South American Midwest by the late Oligocene. We recovered two main lowland and two main Andean clades within the genus; within both Andean clades, most cladogenetic events date back to the Miocene, synchronously with the most intense phase of Andean uplift. In the western clade of lowland *Stenocercus*, species ranges and divergence times are consistent with major landscape shifts at the upper Guaporé and Paraguay River basins as a result of Andean orogeny, suggesting vicariant speciation. By contrast, in the 'horned' lowland clade, we find evidence that dispersal and ecological differentiation have shaped species divergences and current ranges in the Brazilian Cerrado, Caatinga, Pampas and Atlantic Forest, possibly under a vanishing refuge scenario. Lastly, our phylogenetic results indicate two divergent clades within the formerly recognized taxon *S. sinesaccus*, and further evaluation of morphological data corroborates the existence of a distinct, new species of *Stenocercus*, here described. The new taxon occurs in the Chapada dos Parecis massif in the Brazilian states of Mato Grosso and Rondônia.

1. Introduction

Phylogenetic approaches aiming to uncover historical biogeography patterns and the associated drivers of diversification in Neotropical taxa have focused primarily on species from wet forested environments (Carnaval et al., 2009; Costa, 2003; Cracraft and Prum, 1988; Hall and Harvey, 2002; Ribas et al., 2005, 2007). Unfortunately, this focus on wet environments has often resulted in exclusion of species occurring in open and drier biomes from major discussions about the origin of Neotropical biodiversity (Redford et al., 1990; Sánchez-Azofeifa et al., 2005). As a result, early views of a poor and homogeneous biotic composition across the great dry South American diagonal composed of Chaco, Cerrado, Caatinga and Dry Forest biomes have prevailed (Marris, 2005; Vanzolini, 1988, 1994).

More recently, biotic surveys across previously unsampled areas have provided new material for biodiversity studies in open and dry Neotropical settings, supporting biogeographic and

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☆ This paper was edited by the Associate Editor J.A. Schulte.
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http://dx.doi.org/10.1016/j.ympev.2015.09.010
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Please cite this article in press as: Teixeira Jr., M., et al. Molecular data reveal spatial and temporal patterns of diversification and a cryptic new species of lowland *Stenocercus* Duméril & Bibron, 1837 (Squamata: Tropiduridae). Mol. Phylogenet. Evol. (2015), http://dx.doi.org/10.1016/j.ympev.2015.09.010

phylogenetic approaches (Pennington et al., 2004; Nogueira et al., 2011; Werneck et al., 2012; Guedes et al., 2014). This renewed interest has led to unexpected discovery of biodiversity. A noticeable case is that of *Stenocercus* lizards, for which a number of species occurring in open settings has only recently been described and recorded (Nogueira and Rodrigues, 2006; Ribeiro et al., 2009; Torres-Carvajal, 2005). Currently, *Stenocercus* is one of the most speciose South American lizard genera, with 66 valid taxa (Torres-Carvajal, 2007a; Torres-Carvajal and Mafla-Endara, 2013; Venegas et al., 2013, 2014; Köhler and Lehr, 2015). The genus is particularly diverse along the Andes, from Venezuela in the north to central Argentina in the south, reaching up to 4000 m in elevation (Torres-Carvajal, 2007a). Yet, a number of species are also found in the wet Amazonian lowlands, and in the drier, open settings of southern, central and northeastern Brazil (Nogueira and Rodrigues, 2006; Torres-Carvajal, 2007a).

Stenocercus has been addressed by a number of comprehensive systematic revisions (Cadle, 1991; Fritts, 1974; Torres-Carvajal, 2007a). Recently, the implementation of molecular tools and detailed morphological analyses have shed light onto phylogenetic relationships and geographic patterns of diversification within the genus (Torres-Carvajal, 2000, 2007a, 2009; Torres-Carvajal et al., 2006). Importantly, these studies revealed that Andean species compose two main clades, which likely originated in the Central Andes and subsequently dispersed and diversified throughout the Andes. However, because some non-Andean species were described only recently, the full diversity of lowland *Stenocercus* has not yet been addressed in biogeographic studies (e.g., Torres-Carvajal, 2007b; Torres-Carvajal et al., 2006). These gaps have limited our understanding of the temporal and spatial diversification of *Stenocercus* in the open and drier South American lowlands east to the Andes. These species occur in a range of contrasting environments, such as *S. azureus* (Müller, 1880) in the southern Atlantic Forest and Pampas grasslands, *S. quinarius* (Nogueira and Rodrigues, 2006) and *S. squarrosus* (Nogueira and Rodrigues, 2006) in Atlantic dry forests, eastern Cerrado savannas and western Caatinga scrublands, *S. caducus* (Cope, 1862) and *S. sinesaccus* (Torres-Carvajal, 2005) in western Cerrado savannahs, *S. tricristatus* (Duméril, 1851) in southeastern Cerrado savannahs (Torres-Carvajal, 2007a), *S. dumerili* (Steindachner, 1867) in eastern Amazonian forest, and *S. fimbriatus* Avila-Pires, 1995 and *S. roseiventris* (Duméril and Bibron, 1837) in western Amazonian forest.

To improve our understanding about diversification processes in open and dry Neotropical biomes, we provide an updated historical biogeographic scenario of *Stenocercus*, focusing on the genus' diversification to the east of the Andes. We generated new DNA sequence data of previously unsampled *Stenocercus* species, including *S. dumerili*, *S. quinarius*, *S. sinesaccus*, *S. squarrosus*, and *S.* cf. *tricristatus* recently collected in Brazil. By combining these data with existing molecular datasets, we inferred phylogenetic relationships, estimated divergence times, and tracked ancestral distributions, to examine whether cladogenetic events within *Stenocercus* correlate with reported shifts in South American landscapes during the past 30 million years. We also used environmental data to estimate the climatic envelopes currently occupied by lowland *Stenocercus*. We integrated niche and phylogenetic data to test for correlations between genetic and ecological divergence (Graham et al., 2004) and to examine the role of past climatic fluctuations, habitat change and local adaptation in shaping current distribution patterns. Instead of focusing on the better known Andean *Stenocercus*, we emphasize those species from the dry and open lowland settings of South America.

Our new molecular information reveals a highly divergent clade within previously recognized *S. sinesaccus*. As morphological data support that this is a distinct species from the closely related *S. caducus* and *S. sinesaccus*, we formally describe the new taxon herein.

2. Materials and methods

2.1. Molecular analyses

2.1.1. Sampling of molecular data

We generated DNA sequences for 15 specimens: two *Stenocercus caducus*, one *S. doellojuradoi*, four *S. quinarius*, five specimens assigned to *S. sinesaccus*, two *S. squarrosus*, and one *S.* cf. *tricristatus*. We combined our new sequences with GenBank data of several Tropiduridae taxa, including species of *Stenocercus*, *Microlophus*, *Plica*, *Tropidurus*, *Uracentron*, and *Uranoscodon*. Our final dataset comprised 73 samples representing 62 species (Supplementary Table S1).

We extracted total genomic DNA from liver or tail fragments preserved in ethanol using standard protocols. Matching available datasets (Torres-Carvajal, 2007b; Torres-Carvajal et al., 2006), we generated sequences of a long mitochondrial fragment containing the NADH dehydrogenase subunit 1 (ND1) and 2 (ND2) and eight transfer RNAs (tRNA-Ile, tRNA-Gln, tRNA-Met, tRNA-trp, tRNA-Ala, tRNA-Asx, tRNA-Cys, tRNA-Tyr), following Torres-Carvajal et al. (2006). Sequences were edited and aligned using Geneious Pro 6 (Biomatters, Auckland). Models of nucleotide evolution and best-fit partition schemes were determined with Partition Finder v.1.1.1 (Lanfear et al., 2012), implementing PhyML for likelihood estimation (Guindon and Gascuel, 2003) and the Akaike information criterion for model selection (Akaike, 1974).

2.1.2. Inferring phylogenetic relationships

We performed phylogenetic inference under a Bayesian framework using MrBayes 3.2.1 (Ronquist et al., 2012), implementing three independent runs of four Markov chains of 20 million generations each, and sampling every 1000 steps. We partitioned protein-coding genes by codon position as indicated by Partition Finder. Due to the short length of each of the eight tRNAs (~70 base pairs), we treated them as a single partition (631 base pairs total). We assessed convergence and stationarity of model parameters using Tracer 1.5, combined runs in LogCombiner 1.8 (with 25% discarded as burn-in), and summarized a maximum clade credibility tree in TreeAnnotator 1.8 (Drummond et al., 2012). We unlinked parameters of substitution rates and nucleotide frequencies between partitions. The resulting topologies were visualized in FigTree 1.4 (available from http://tree.bio.ed.ac.uk/software/figtree/).

For descriptive purposes, we also estimated Tamura–Nei corrected pairwise genetic distances (Tamura and Nei, 1993) for the mitochondrial DNA fragment of all populations assigned to *Stenocercus sinesaccus* and *S. caducus*, using the APE 3.1 package (Paradis et al., 2004) of the R 3.0.2 platform (R Core Team, 2015).

2.1.3. Divergence time estimation

To estimate divergence times between *Stenocercus* species, as well as between the genus and related Tropiduridae taxa, we performed simultaneous phylogenetic reconstruction and divergence time estimation using BEAST v.1.8 (Drummond et al., 2012). To calibrate the root of Tropiduridae, we set a normally-distributed prior on the node corresponding to the most recent common ancestor of *Stenocercus*, *Uranoscodon* and *Plica* (mean = 49 Mya, standard deviation = 5.1 Mya) following Prates et al. (2015). We rooted our tropidurid tree by implementing a molecular clock strategy, which yields rooted trees in the absence of outgroups through the simultaneous estimation of tree topology and branch lengths (Felsenstein, 2004; Drummond and Rambaut, 2007). Molecular clock-based rooting has been found to be a robust and effective method (Huelsenbeck et al., 2002), especially useful in cases where an outgroup is not available or where deep divergence between the

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