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Gene and species trees of a Neotropical group of treefrogs: Genetic diversification in the Brazilian Atlantic Forest and the origin of a polyploid species

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ABSTRACT

The Neotropical *Phyllomedusa burmeisteri* treefrog group includes four diploid (*P. bahiana*, *P. burmeisteri*, *P. distincta* and *P. iheringii*) and one tetraploid (*P. tetraploidea*) forms. Here we use mitochondrial and nuclear sequence variation from across its range to verify if recognized morphospecies correspond to phylogenetic clades, examine the origin of the polyploid *P. tetraploidea*, and compare range wide patterns of diversification to those of other BAF organisms. We compared single gene trees with one Bayesian multi-gene tree, and one Bayesian species tree inferred under a coalescent framework. Our mtDNA phylogenetic analyses showed that *P. bahiana*, *P. burmeisteri* and *P. iheringii* correspond to monophyletic clades, while *P. distincta* and *P. tetraploidea* were paraphyletic. The nuclear gene trees were concordant in revealing two moderately supported groups including (i) *P. bahiana* and *P. burmeisteri* (northern species) and (ii) *P. distincta*, *P. tetraploidea* and *P. iheringii* (southern species). The multi-gene tree and the species tree retrieved similar topologies, giving high support to the northern and southern clades, and to the sister-taxa relationship between *P. tetraploidea* and *P. distincta*. Estimates of ^{t}MRCA suggest a major split within the *P. burmeisteri* group at $\approx$5 Myr (between northern and southern groups), while the main clades were originated between $\approx$0.4 and 2.5 Myr, spanning the late Pliocene and Pleistocene. Patterns of geographic and temporal diversification within the group were congruent with those uncovered for other co-distributed organisms. Independent paleoecological and geological data suggest that vicariance associated with climatic oscillations and neotectonic activity may have driven lineage divergence within the *P. burmeisteri* group. *P. tetraploidea* probably originated from polyploidization of *P. distincta* or from a common ancestor.

1. Introduction

The Brazilian Atlantic Forest (BAF) has lost around 90% of its original area of more than one million square kilometers (Morellato and Haddad, 2000; Ribeiro et al., 2009). BAF is however among the most biodiverse regions of the world, harbouring one of the largest percentages of endemic species (Myers et al., 2000). The evolutionary processes that led to this extraordinarily diverse biome have long intrigued evolutionary biologists, but there are only a few studies unveiling the evolutionary processes that determined biological diversification in this megadiversity hotspot. Recently, some molecular studies have confirmed theoretical predictions that species' biogeographical histories have been impacted by the recurrent isolation and persistence of organisms in forest refugia throughout Quaternary climatic fluctuations – Pleistocene refugia hypothesis (*sensu* Haffer, 1969) – (e.g. Cabanne et al., 2007; Carnaval et al., 2009). Other studies, however, suggested that not only Quaternary climatic fluctuations but also climatic and geologic factors such as major rivers, mountain chains and Tertiary tectonic events associated with the formation of geographical landmarks determined current patterns of genetic diversity and diversification of many BAF taxa (Lara and Patton, 2000; Grazziotin et al., 2006; Pellegrino et al., 2005, but see review in Rull, 2008). Forest refugia and geographic barriers could have also acted in combination to produce taxon-specific idiosyncratic patterns of diversification (Grazziotin et al., 2006; Cabanne et al., 2008). To understand their relative importance in producing biological diversification will require detailed multi-species phylogeographical studies across organisms with distinct life histories. This will be especially relevant given that complex topography shaping the diverse phytophisiognomies of the BAF (Oliveira–Filho and Fontes, 2000).

Recent studies analyzing both molecular data and palaeoclimatic models of the BAF (Carnaval and Moritz, 2008; Carnaval et al., 2009) suggested spatial variation in forest persistence throughout the Pleistocene, predicting a large area of historical forest stability

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in the central corridor (Bahia) and in other small areas (Pernambuco) along the Brazilian coast, roughly concordant with current centres of endemism and mtDNA diversity patterns of few taxa (Costa et al., 2000; Costa, 2003; Cabanne et al., 2008), including three BAF treefrog species (Carnaval et al., 2009). Because the species of the *Phyllomedusa burmeisteri* group are widespread mostly in the BAF, inhabiting submontane to montane areas of rainforest, mixed ombrophilus, semi-deciduous and deciduous forest, they are appropriate model organisms for understanding diversification mechanisms and biogeographical patterns across the BAF. Five species are currently recognized within the *P. burmeisteri* species group based on external morphology, vocalization, and cytogenetics (Pombal and Haddad, 1992; Silva-Filho and Juncá, 2006): the diploid *P. burmeisteri*, *P. bahiana*, *P. distincta*, *P. iheringii*, and the tetraploid species *P. tetraploidea*. Their parapatric or allopatric species ranges replace each other along the BAF northeastern to southern Brazil, with the southernmost *P. iheringii* reaching the Uruguayan Pampas (Fig. 1). The tetraploid species is the only with an exclusively inland distribution in southern BAF, including areas in Argentina, Brazil and Paraguay. These treefrogs occur mainly in forested habitats of the BAF, breeding in ponds near the forest edge. The southern *P. iheringii* is an exception as it may occur and breed in the more open areas of the Pampas biome. Although the morphospecies occur mostly in non-overlapping distributions, areas of phenotypic intergradation between *P. bahiana* and *P. burmeisteri*, and one area of natural hybridization between *P. distincta* and *P. tetraploidea* were previously recognized (Pombal and Haddad, 1992; Haddad et al., 1994).

Phylogenetic relationships across the subfamily *Phyllomedusinae* based on several mitochondrial and slowly-evolving nuclear genes (exons) supported the monophyly of described *P. burmeisteri* morphospecies, but showing only moderate support to the sister-taxa relationship of *P. tetraploidea* with *P. distincta* (Faivovich et al., 2009). Because those results relied on a parsimony analysis of a concatenated dataset, including few representatives per species, were here undertake a rangewide more inclusive multilocus statistical phylogenetic analyses using both mitochondrial and more variable nuclear gene introns under a coalescent framework, to examine species diversification within the *P. burmeisteri* group, particularly the origin of *P. tetrapoidea*.

Speciation through polyploidization, once considered relatively rare in animals, has been reported for several vertebrates including

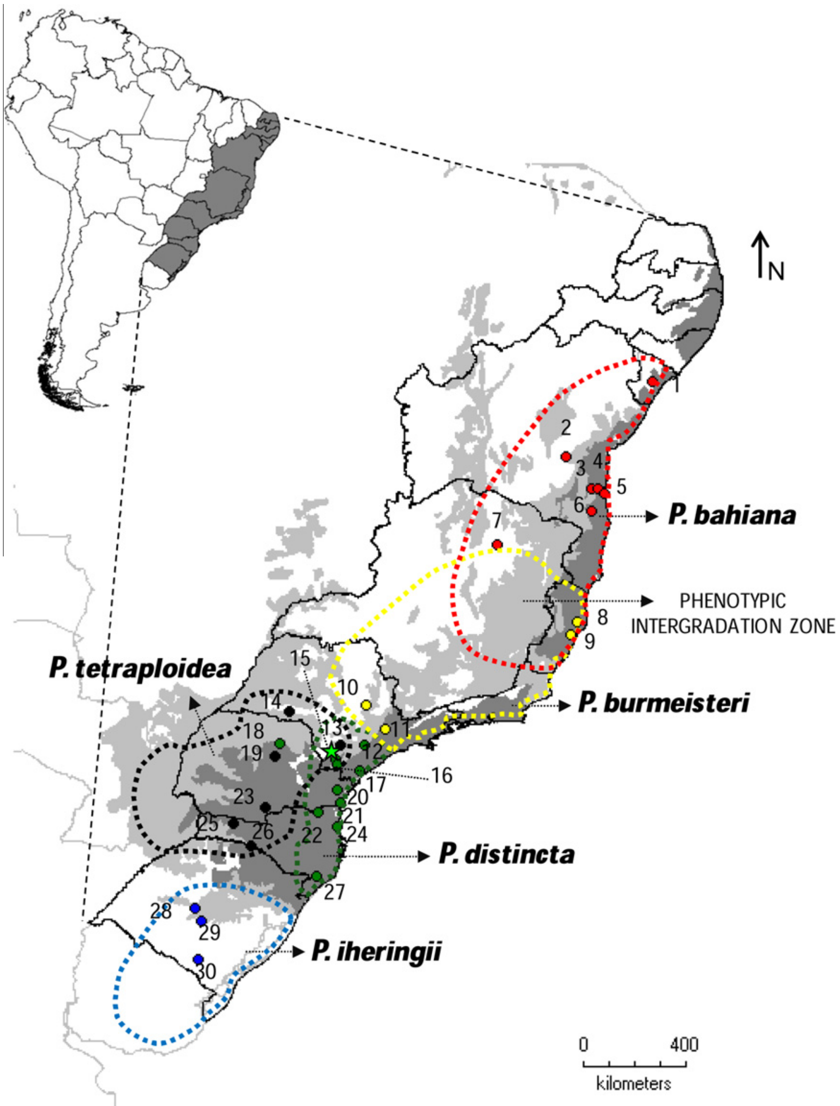

Fig. 1. Geographic distribution of *Phyllomedusa burmeisteri* species group with sampling localities numbered 1–30. Brazilian Atlantic Forest original cover: ombrophylous (dark gray) and semi-deciduous (light gray) forests are represented. The green star marks the reported hybridization between *Phyllomedusa distincta* and *Phyllomedusa tetraploidea*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)